University of Alberta

Interaction of Dopamine and Glutamate in the Context of Reward

by

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fulfillment of the requirements for the degree of Doctor of Philosophy

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There are two modes of acquiring knowledge, namely by reasoning and experience. Reasoning draws a conclusion and makes us grant the conclusion, but does not make the conclusion certain, nor does it remove doubt so that the mind may rest on the intuition of truth, unless the mind discovers it by the path of experience.

– Roger Bacon (c. 1214-1292)

Dedication

This thesis is for my family: past, present, and future.

Abstract

Dysfunction in the balance of dopamine (DA) and glutamate (Glu) in the brain pathway from the ventral tegmental area (VTA) to the nucleus accumbens (NAS) may play a role in human disorders of motivation, such as schizophrenia and drug abuse. This thesis assessed DA-Glu interactions using objective measurements of motivated behaviour in laboratory rats, including VTA brain stimulation reward (BSR), conditioned place preference (CPP), and spontaneous locomotor activity. Male Sprague-Dawley rats were administered drugs systemically or directly into the NAS shell subregion. For BSR experiments, rate-frequency thresholds were assessed independently of drug effects on performance. CPP was assessed using a two-compartment conditioning procedure, while drug effects on spontaneous locomotor activity were monitored using photocell locomotor activity boxes. Effects of the Glu NMDA receptor antagonist MK-801 (dizocilpine) or the Glu AMPA/kainate receptor antagonist NBQX were assessed alone or combined with the DA agonists 7-OH-DPAT or apomorphine. The main findings were: 1) MK-801 facilitated BSR whether given systemically or directly into the NAS shell, while NBQX was ineffective; 2) low doses of 7-OH-DPAT or apomorphine decreased BSR; 3) reward-enhancing effects of MK-801 were additive with effects of DA agonists; 4) intra-NAS NBQX and 7-OH-DPAT alone were ineffective, but when combined decreased BSR; 5) a high dose of 7-OH-DPAT induced a CPP that was blocked by co-administration of MK-801 during conditioning, without conditioned locomotor effects; 6) low doses of 7-OH-DPAT or apomorphine alone induced hypoactivity while systemic or intra-NAS shell

MK-801 had opposite effects; and 7) 7-OH-DPAT effects demonstrated significant interaction with MK-801, while apomorphine effects did not. While effects of Glu NMDA receptor blockade may be additive with effects of DA agonists in BSR, locomotor stimulation induced by Glu NMDA receptor antagonism is blocked by postsynaptic D_3 DA receptor stimulation, but not by stimulation of D_2-like DA autoreceptors. Taken together, these data provide additional evidence for "behaviourally inhibitory" D_3 DA receptors that may be located postsynaptically in the NAS. As dysfunction in the balance of DA-Glu systems may underlie the pathophysiology of drug abuse and schizophrenia, the current results may be clinically relevant to the development of potential pharmacotherapeutics.

Preface

Many budding scientists are drawn into neuroscience research due to their fascination or curiosity with the complexity of the mind and brain. Research on one aspect of this wonderful tissue, referred to as 'psychopharmacology' or 'neuropsychopharmacology', deals with the uses or effects or modes of action of drugs on the mind or brain ('neuro-' from the Greek *neuron* or νευρον meaning 'nerve'; 'psycho-' from the Greek *psukho* or ψχο meaning 'breath, life, or soul'; 'pharmaco-' from the Greek *pharmakon* or φαρμαχο meaning 'drug'; '-logy' from Greek *logos* or λογοσ meaning 'word or reason'). In other words, neuropsychopharmacology can be considered the reasoning or study of the effects of drugs on the nervous system and the psyche. Over the past few years, with the guidance of my supervisor, I have conducted a series of experiments that logically explore the interaction between two important neurotransmitters in the context of reward. Specifically, we have taken a closer look at the neurotransmitters dopamine and glutamate to determine how these agents interact in the intact mammalian brain, using the rodent as the subject of study. When one investigates in the area of 'behavioural pharmacology' it is necessary to decide which behaviours will be measured; for the purposes of this thesis, we have focused on investigating drug effects using three types of experiments: measurement of spontaneous locomotor activity (movement of the rat about a square box); measurement of place conditioning (pairing drug effects with a distinct environment to see if the rat 'prefers' the environment in which it received the drug); and measurement of brain stimulation reward, or intracranial electrical self-stimulation, in which the rat receives electrical stimulation to a specific brain region after it performs some task, such as pressing a lever.

The approach used in this thesis was to investigate the interaction between dopamine and glutamate using drugs that target specific receptor subtypes, and to use the same compounds in a variety of behavioural measurements. In the end, as the thesis progresses from one experiment to the next, the reader should gain an appreciation for the thickening plot, and will hopefully appreciate how the research presented here relates to our ability to understand and develop new and improved treatments for various psychiatric illnesses.

Acknowledgements

Many people contributed greatly to the successful completion of my graduate program. First and foremost, I am indebted to my parents, Fred and Lorraine Clements of Prince Edward Island, for providing me the opportunity (and desire) to pursue post-secondary education. My fiancée, Mia Biondo, offered her unconditional support through thick and thin (and even none!). Without doubt, she has helped to keep it all in perspective from our very first day in the lab together. My thesis supervisor and mentor, Andy Greenshaw, offered his steadfast support and continuous generosity, complete with many conferences and eye-opening dinners! Banff, Fernie, Ottawa, Montréal, and Kingston were delightful (though I now regret declining the opportunity to attend IBRO in Prague).

I must also acknowledge my immediate and extended family members. The holiday seasons and summer vacations would not be complete without their curious inquisitions into my progress and future prospects; this actually helped to keep everything on track.

Many student colleagues brightened the corridors at school and the streets of Edmonton. I especially value the true friendships with Kwang-Ho Choi, Emily Gordon, and Tera Mosher, who have each helped me more than they know. I also appreciate the impromptu guidance and contributions to histological results provided by student colleagues David Hayes and Melissa Kelly, as well as members of the John Greer and Chris Sturdy laboratories.

Several professors have helped direct my program by participating in my examining committees and offering constructive feedback, including Andy

Greenshaw, Glen Baker, Peter Silverstone, the late Douglas Grover, Clayton Dickson, Susan Dunn, and Kathryn Todd. I am especially thankful for the support provided by Andy Greenshaw, Glen Baker, and Deborah James; in addition to teaching me about role models, they undoubtedly shaped my professional future.

My program benefited from superb administration provided by Carol Ann Johnson in Neuroscience and Pips Wolfaardt in Psychiatry. In addition, lab technicians Rick Strel, Gail Rauw, and Jordyce Van Muyden were helpful with everyday logistics. Kathryn Todd and Satya Kar provided not only the space but also the expertise that enabled my learning of basic histology.

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List of Symbols, Nomenclature, and Abbreviations

°C	degree(s) Celsius
5-HT	5-hydroxytryptamine; serotonin
7-OH-DPAT	(±)-7-hydroxy-2-dipropylaminotetralin
AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
AMYG	amygdala
ANOVA	analysis of variance
AP	anterior-posterior
AP5	2-amino-5-phosphonopentanoic acid (also APV)
APO	apomorphine
APV	2-amino-5-phosphonovaleric acid (also AP5)
BSR	brain stimulation reward; intracranial self-stimulation
cAMP	cyclic adenosine monophosphate
CB	cannabinoid
CC	corpus callosum
CLMA	conditioned locomotor activity
cm	centimeter(s)
CNQX	6-cyano-7-nitroquinoxaline-2,3-dione
CNS	central nervous system
CoA	coenzyme A
COMT	catechol-O-methyltransferase
CP	caudate/putamen
CPA	conditioned place aversion

CPP	conditioned place preference
CSF	cerebrospinal fluid
DA	dopamine
DNQX	6,7-dinitroquinoxaline-2,3-dione
DOPAC	3,4-dihydroxyphenylacetaldehyde
DV	dorsal-ventral
EEDQ	N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline
fMRI	functional magnetic resonance imaging
g	gram(s)
GABA	γ-aminobutyric acid
Glu	glutamate
GP	globus pallidus
HIP	hippocampus
HVA	homovanillic acid
Hz	hertz
IAZ	interaural zero
ICV	intracerebroventricular
IP	intraperitoneal
IV	intravenous
kg	kilogram(s)
L-DOPA	L-3,4-dihydroxyphenylalanine
LH	lateral hypothalamus
LM	lateral-medial

M	molar
M_{50}	rate-frequency threshold
MAO	monoamine oxidase
MFB	medial forebrain bundle
mg	milligram(s)
Mg^{2+}	magnesium ion(s)
mGluR	metabotropic glutamate receptor
min	minute(s)
MK-801	(5S,10R)-(+)-5-methyl-10,11-dihydro-5H-dibenzo[a,d]cyclophepten-5,10-imine maleate; dizocilpine
mL	milliliter(s)
mm	millimeter(s)
mM	millimolar
mPFC	medial prefrontal cortex
MRI	magnetic resonance imaging
ms	millisecond(s)
NAS	nucleus accumbens septi
NBQX	2,3-dioxo-6-nitro-1,2,3,4-tetrahydrobenzo[f]quinoxaline-7-sulfonamide
ng	nanogram(s)
NMDA	N-methyl-D-aspartic acid
OT	olfactory tubercle
PBS	phosphate buffered saline

PCP	phencyclidine
PET	positron emission tomography
PFC	prefrontal cortex
pH	negative logarithm of hydrogen ion concentration
RMAX	maximum response at a single frequency
s	second(s)
SC	subcutaneous
SN	substantia nigra
TH	thalamus
TRES	total number of responses per session
μA	microampere(s)
μg	microgram(s)
μL	microlitre(s)
μm	micrometer(s)
UPC	unbiased place conditioning
VP	ventral pallidum
VTA	ventral tegmental area

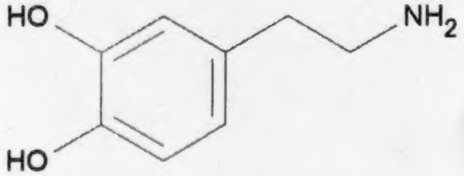
Chapter 1. Introduction

Evidence from both human and animal studies indicates that the balance between the neurotransmitter dopamine (DA) and the excitatory amino acid neurotransmitter glutamate (Glu) in the brain may affect state of arousal or motivation. In addition, a wealth of studies has detected abnormal DA and Glu functioning in patients that demonstrate dysfunction in motivation due to schizophrenia or chronic exposure to drugs of abuse (for review, see Soares and Innis 1999). The aim of this thesis was to explore DA-Glu interactions in this context by assessing changes in reinforcement or arousal in rats following administration of pharmacologically selective drugs, systemically or directly into the forebrain. Below, following brief reviews of the relevant pharmacology of DA and Glu, the potential interaction between these neurotransmitters is discussed based upon data from both human and animal studies. This introductory chapter concludes by briefly describing the behavioural methodology used in this thesis.

Dopamine

As reviewed by Cooper et al. (2003), the aromatic amino acid *para*-tyrosine is derived mostly from dietary protein and is converted to L-3,4-dihydroxyphenylalanine (L-DOPA) by tyrosine hydroxylase; this is the rate-limiting enzyme in catecholamine synthesis. L-DOPA is decarboxylated to produce the catecholamine DA (Figure 1.1), a precursor of norepinephrine and epinephrine (also called noradrenaline and adrenaline respectively). Collectively, these neurotransmitters are called the catecholamines as they contain a benzene ring with two adjacent hydroxyl substituents (-OH) with a single amine ($-NH_2$), as shown in the example below. The activity of catecholamines in the synapse is terminated by either reuptake into the presynaptic nerve terminal by cell membrane transporters, or by catabolism. The degradation of the DA may occur by two alternate paths that involve the enzymes monoamine oxidase (MAO) and catechol-O-methyltransferase (COMT) to yield the major metabolites 3,4-dihydroxyphenylacetaldehyde (DOPAC) and homovanillic acid (HVA). Some studies suggest that up to 80% of total brain catecholamine content may be attributed to DA (Feldman et al. 1997).

Figure 1.1. Structure of DA.



Generally, receptors for neurotransmitters such as catecholamines are ligand-binding molecules located on the cell surface. It has been known for over 25 years that receptors for DA may be classified into two distinct categories: D_1

and D_2. Further work has revealed three additional subtypes (D_3, D_4, and D_5) that have since been cloned and characterized (Sokoloff et al. 1990; Bouthenet et al. 1991; Van Tol et al. 1991; Sunahara et al. 1991; Schotte et al. 1992; Diaz et al. 2000). All of the known DA receptors are metabotropic (coupled to G proteins) and are currently subdivided into the D_1-like (D_1 and D_5) and D_2-like (D_2, D_3, and D_4) families, based upon stimulation or inhibition of adenylyl cyclase (and related cAMP formation) respectively.

Ligands targeting the family of D_2-like DA receptors are used in this thesis. The D_2 DA receptor subtype exists in two isoforms (long and short; D_{2L} and D_{2S}) based upon additional amino acids found in the third cytoplasmic loop of the receptor. It is important to note that in addition to signal transduction by inhibition of cAMP formation, D_2 DA receptors may also activate potassium channels, leading to membrane hyperpolarization and decreased neuronal excitability (Cooper et al. 2003). Less is known about D_3 DA receptors, likely due to the lack of truly selective agonists and antagonists for this receptor subtype. However, the D_3 DA receptor subtype is of primary interest in this thesis because of its abundant expression in brain regions associated with motivation and reinforcement (this is discussed further below).

Dopamine receptors may be located on the postsynaptic neuron, or on the terminals, soma, or dendrites of presynaptic DA neurons; the latter are referred to as DA autoreceptors. Stimulation of DA terminal autoreceptors inhibits the synthesis and release of DA; stimulation of DA autoreceptors on the soma or on dendrites of the presynaptic neuron may reduce cell firing and, in turn, reduce terminal DA release (Adell and Artigas 2004). The latter autoreceptors modulate cell activity and DA release through the release of DA from dendrites (Santiago and Westerink 1991). Whereas DA autoreceptors affect DA release via activity of K^+ channels, inhibition of DA synthesis by DA autoreceptors may be related to reduced cAMP-dependent activation of tyrosine hydroxylase (Strait and Kuczenski 1986; Onali et al. 1992).

The distribution of DA receptors in the brain has been characterized using autoradiography of subtype-specific ligands (see Table 1.1). Of primary interest in this thesis is the localization of D_2 and D_3 DA receptors to the nucleus accumbens septi (NAS) (Mengod et al. 1992; Sokoloff et al. 1992).

Despite commonalities in origins and terminal fields, it is generally accepted that there are four major DA pathways in the brain; a simplified summary is shown in Table 1.2. Of primary interest in the context of reward and reinforcement is the mesolimbic dopaminergic projection that originates in the ventral tegmental area (VTA) and terminates in the NAS (this is discussed further below). The development and degeneration of dopaminergic neurons was recently reviewed by Chinta and Andersen (2005).

Table 1.1. Summary of DA receptor subtypes. Modified from Cooper et al. (2003).

Family	Sub-type	Major expression in these brain areas	Effect	Agonist	Antagonist
D_1-like	D_1	CP, NAS, OT, AMYG	↑ cAMP	SKF 38393	SCH 23390
	D_5	HIP, TH			
D_2-like	D_2	SN, CP, NAS, OT	↓ cAMP	Bromocriptine	Haloperidol; Sulpiride
	D_3	Islands of Calleja; NAS; OT		7-OH-DPAT	UH 232
	D_4	Frontal cortex; hypothalamus		CP 226269	Clozapine

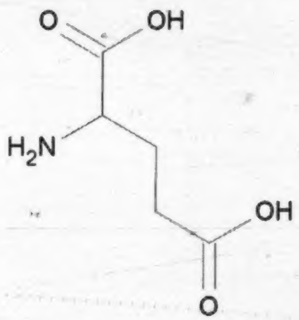
Table 1.2. Major DA pathways in the brain.

Pathway	Location	Generalized Function
Nigrostriatal	SN into dorsal striatum (CP, GP)	Motor functions
Mesocortical	VTA to PFC, cingulate, entorhinal areas	Learning, memory
Mesolimbic	VTA to NAS, OT, AMYG	Reward, motivation
Tuberoinfundibular	Arcuate and periventricular nuclei into the intermediate lobe of the pituitary and into the median eminence	Prolactin release

Glutamate

The nonessential amino acid glutamate (glutamic acid; Glu) is an abundant neurotransmitter found ubiquitously in the mammalian central nervous system (CNS) (Figure 1.2). Based on its abundance, and the observation that neurons are strongly depolarized by iontophoretic application of Glu, this amino acid is considered to be a principal transmitter for excitatory signaling in the CNS. Glu is also the main precursor of the major inhibitory neurotransmitter γ-aminobutyric acid (GABA).

Figure 1.2. Structure of Glu.



As reviewed by Cooper et al. (2003), Glu is synthesized through two main pathways in neural tissue. Most Glu is produced by the oxidative metabolism of glucose to acetyl coenzyme A (CoA). In the citric acid cycle, acetyl CoA is converted to α-ketoglutarate that is then transaminated to Glu. In a second pathway, the glutamine cycle, Glu is formed from glutamine; however this pathway does not result in any *net* production of Glu as glutamine itself is formed from Glu. The glutamine cycle is mainly a pathway for temporary storage of Glu in non-neural connective tissue such as astrocytes or oligodendrocytes (glia). In the synapse, Glu is removed by uptake into presynaptic nerve terminals or glia via a Glu transporter. The mechanisms for degradation of Glu resemble the pathways of synthesis; Glu may be oxidatively deaminated to form α-ketoglutarate, or Glu may be inactivated by conversion to glutamine in astrocytes.

There are currently four known classes of Glu receptors organized into ionotropic and metabotropic families. Ionotropic Glu receptors, belonging to the ligand-gated ion channel receptor family, are defined by the depolarizing excitatory actions of selective agonists. N-Methyl-D-aspartic acid (NMDA), α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), and kainate receptors have been identified; AMPA and kainate receptors are collectively referred to as non-NMDA receptors (see Table 1.3). The activity of NMDA receptor channels is voltage-dependent; at resting membrane potential, the channel is blocked by relatively low concentrations of magnesium ions (Mg^{2+}). At this membrane potential, NMDA agonists are generally ineffective; agonist efficacy increases when the cell is depolarized. It may be that depolarization provided by stimulation of non-NMDA receptors enables NMDA receptor function. The NMDA receptor complex is currently known to have distinct binding sites for the following: L-Glu, glycine, phencyclidine(PCP)/MK-801, magnesium ions (Mg^{2+}), zinc ions, and the polyamines spermine and spermidine.

The multiple regulatory sites and ligand-binding abilities of the NMDA receptor complex may account for its widespread associations with several neurological and pathological processes.

There are currently eight distinct types of metabotropic Glu receptors (mGluRs) that are coupled to various second messenger systems via G proteins. Based on amino acid sequences and receptor pharmacology, mGluRs are classified into three families: group I ($mGluR_1$ and $mGluR_5$) are coupled to phosphatidylinositol hydrolysis (i.e., to stimulate inositol phosphate metabolism) and mobilize intracellular calcium via phospholipase C. In contrast, group II ($mGluR_2$ and $mGluR_3$) and group III ($mGluR_4$, $mGluR_6$, $mGluR_7$, $mGluR_8$) decrease adenylyl cyclase and reduce cAMP formation.

Table 1.3. Summary of Glu receptor classes

Class	**Subunits**	**Effect**	**Agonist**	**Antagonist**
NMDA	NR1, NR2A-D	Na^+, K^+, Ca^{2+}	NMDA	MK-801
AMPA	GluR1-R4	Na^+, K^+	AMPA	NBQX
KA	GluR5-7, KA1-2	Na^+, K^+	Kainate	CNQX
Metabotropic	Subtypes mGluR1-mGluR8	IP_3, cAMP, DAG	Classified by groups I-III	

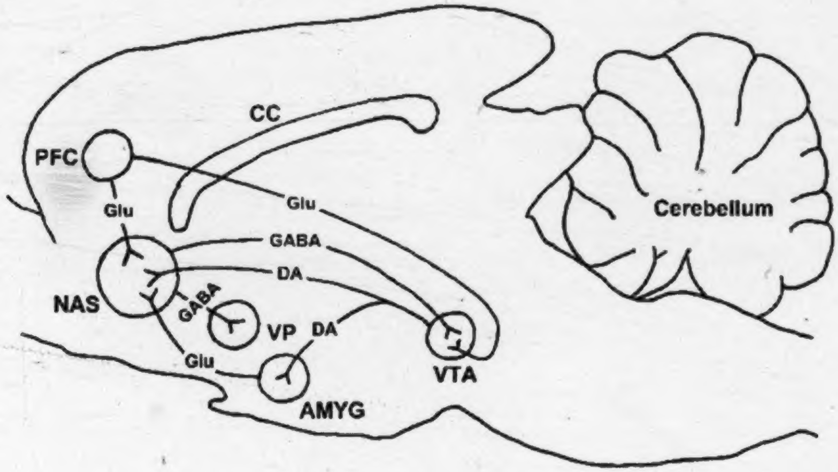
Brain circuitry of reward and reinforcement

Before describing how this thesis examined the neural circuitry associated with reward, a brief review of the relevant anatomical systems is useful. As described above, the mesolimbic circuit is comprised of DA-containing cell bodies in the VTA that terminate in the NAS. The interaction between DA and Glu in this context is very complex and this thesis will attempt to simplify the interaction for readability.

The VTA is located in the A10 region of the ventromedial mesencephalon; it is populated mainly by DA-containing neurons that project to the NAS; however a subpopulation of VTA neurons that project to the NAS is GABAergic (Van Bockstaele and Pickel 1995). It has been shown that both the VTA and NAS receive afferents from many brain regions; while the VTA primarily receives input from the PFC (Glu) and NAS (GABA), the NAS receives input from the VTA (DA) and PFC (Glu) (Taber et al. 1995; Ikemoto and Panksepp 1999).

It is generally accepted that Glu input to the VTA stimulates DA cell activity to facilitate DA release in the NAS. In the NAS, Glu may also facilitate DA release by binding to presynaptic DA terminals. There may be a reciprocal relationship between DA and Glu (see Morari et al. 1998) in that Glu transmission may be affected by DA via feedback mechanisms (Imperato et al. 1990; Yang and Mogenson 1987). Specifically, it has been proposed that in the NAS, dopaminergic and glutamatergic inputs may innervate postsynaptic medium spiny neurons in close proximity; these neurons are presumably GABAergic and provide reciprocal inhibitory feedback to alter cell firing in the VTA (Sesack and Pickel 1992; Gracy and Pickel 1996). A similar relationship in the cortex has been coined a triadic synaptic arrangement (Goldman-Rakic 1992), and a simplification of this synaptic arrangement is shown in Figure 1.3.

Figure 1.3. Neurotransmitter systems related to mesolimbic DA. Adapted from Ikemoto and Panksepp (1999) and Feldman et al. (1997).



Since the NAS is the major brain region explored in this thesis, additional information about this area is warranted. The NAS is located bilaterally within the ventral striatum and has been proposed to act as an interface between limbic circuits and motor circuits (Mogenson et al. 1980); in other words, it is postulated that the NAS is critically involved in an organism's motor responses to the processing of sensory information, which is the foundation of reinforcement.

The NAS may be divided into two major subregions, the ventromedial shell and the dorsolateral core (Voorn et al. 1986; Voorn et al. 1989; Meredith et al. 1989; Heimer et al. 1991; Meredith et al. 1992; Pennartz et al. 1992; Zahm and Brog 1992; Zahm 1999). These subregions may be functionally distinct based on pharmacological (Deutch and Cameron 1992; Robledo and Koob 1993), electrophysiological (Rebec et al. 1997), and behavioural observations (Koshikawa et al. 1996; Kitamura et al. 1999). NAS subregions also demonstrate different afferent/efferent projections (for review see Brog et al. 1993; Groenewegen et al. 1999). The NAS shell projects to many areas, including the VTA, the lateral hypothalamus, and extended amygdala (which includes the centromedial amygdala and bed nucleus of the stria terminalis) (Heimer et al. 1991; Ikemoto and Panksepp 1999). In addition, evidence from studies of microdialysis and voltammetry indicates that drugs of abuse may increase extracellular DA in the NAS shell subregion (Pontieri et al. 1995; Pontieri et al. 1996; Nisell et al. 1997). As such the NAS shell may be predominantly involved in limbic function rather than the NAS core (Deutch et al. 1993); however recent evidence may also implicate the NAS core in reward processes (Hatip-Al-Khatib et al. 2001; Cardinal et al. 2004; Li et al. 2004; Giertler et al. 2005). In addition, the NAS subregions may be further subdivided; there is evidence to suggest that there exists a functional rostral-caudal dichotomy within the NAS shell subregion

(Heidbreder et al. 1999). However, exploitation of this knowledge is restricted by the limited precision of our current microinjection procedures.

It is noteworthy that the ventral pallidum (VP) has also been implicated in reward-related neural mechanisms, especially in relation to opioids and psychostimulants (see Skoubis and Maidment 2003). This brain area is innervated by GABAergic interneurons that originate in the NAS; it has therefore been proposed that this pathway may transfer information from the mesolimbic system to motor-related brain areas (Koob and Swerdlow 1988) to affect goal-directed behaviour.

Dopamine and Glutamate in Psychiatry

The interaction between DA and Glu in the mesolimbic system is a primary focus of research into understanding the mechanisms of drug addiction and reward-related learning. Dysfunction of DA and Glu systems in this area of the brain has been associated with many psychiatric disorders, and in addition, the neurodegeneration associated with Parkinson's and Alzheimer's diseases. In the clinical psychiatric realm, evidence suggests that DA and Glu may contribute to the underlying mechanisms of substance abuse, schizophrenia, depression, disorders of anxiety, and attention-deficit disorders. As such, a brief review of the current status of DA and Glu in the prevalent disorders of drug abuse and schizophrenia is useful.

Drug Abuse

Drug abuse is a very complex neurological and behavioural disorder that is generally typified by compulsive drug-seeking behaviours. Addictive behaviour is likely a culmination of both primary (unconditioned) and secondary (conditioned) reward that is affected by learning and memory processes, sensitization, withdrawal, and relapse (Weiss 2005).

Historically, addiction research has focused on alteration of DA transmission. Wise and Bozarth (1987) proposed that the reinforcing effects of abused drugs are due to the facilitation of DA transmission in the NAS. It was subsequently shown that many drugs of abuse (e.g., amphetamine, nicotine, cocaine, morphine, and alcohol) increase DA turnover in the NAS as measured by in vivo microdialysis, presumably by stimulating cell firing in the VTA and increasing DA release downstream in the NAS (Di Chiara and Imperato 1988). In relation to DA receptors, Le Foll et al. (2005) have recently shown that acute cocaine administration induced a long-lasting increase in the expression of D_3 DA receptors in the NAS shell subregion. This finding underscores the importance of investigating the role of D_3 DA receptors in response to drugs of abuse. However, it is increasingly clear that DA is not the only neurochemical mediating addiction; dopaminergic medications have (so far) made little contribution to the effective treatment of substance abuse. To that effect, a wide variety of pharmacotherapies are being evaluated for the treatment of cocaine addiction, including medications that target GABA, adrenoceptors, or tissue plasminogen activator, and other medications such as vasodilators and vaccines in an attempt block cocaine's penetration into the brain (Sofuoglu and Kosten 2005; Yamada et al. 2005).

Evidence from studies of the neural circuitry in the NAS (described above) suggests there is modification of the mesolimbic DA system by Glu. Studies of behaving animals in models of addiction support this hypothesis. For example, intra-NAS injection of AMPA may induce relapse to drug-seeking (Cornish and Kalivas 2000). In addition, Glu antagonists in the NAS may block cocaine-induced relapse to drug-seeking (see Chapter 2). Recently, Kalivas et al. (2005) have suggested that the excitatory Glu projection from the PFC to the NAS may underlie drug-seeking; in other words, adaptations in Glu release at synapses in the NAS originating from the PFC may result in compulsive focusing on drug-associated cues.

Glu may also be involved in the mechanisms of drug abuse independently of DA. One study that used mice lacking mGluR5 receptors showed a lack of reinforcing effects of cocaine as this drug was not self-administered in these mice (Chiamulera et al. 2001). This effect occurred while baseline DA function was normal and cocaine continued to stimulate DA release.

There is also evidence to suggest that drugs of abuse may affect receptor expression in the NAS. For example, the NAS exhibits a high expression of mGluR5 receptors (Tallaksen-Greene et al. 1998) and repeated administration of cocaine increases the expression of mGluR5 in the NAS shell (Ghasemzadeh et al. 1999). In addition, cocaine and morphine may increase expression of both

NMDAR1 (an NMDA receptor subunit) and GluR1 (an AMPA receptor subunit) in the VTA (Fitzgerald et al. 1996; Churchill et al. 1999). Lastly, studies of brain stimulation reward (an experimental paradigm that resembles addiction – see below) have shown that electrical self-stimulation of the medial forebrain bundle (MFB) decreases expression of the GluR1 subunit in the VTA (Carlezon et al. 2001).

This brief review of DA-Glu interaction in the context of drug abuse indicates that the relationship between these neurotransmitters in the mesolimbic system is important for understanding the mechanisms of drug addiction. In a similar clinical context, the balance of DA and Glu in the midbrain may also be important for our understanding of the neural dysfunctions related to schizophrenia.

Schizophrenia

Schizophrenia is a medical illness that typically begins to affect young men and women between the ages of 16 and 30. According to the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV), schizophrenia is characterized by both "positive" and "negative" symptoms. Positive symptoms are those including psychosis (hallucinations and delusions/paranoia), disorganized thoughts, and catatonia. Hallucinations may be based upon any of the senses and can be classified as auditory, visual, tactile, or olfactory; delusions may be grandiose, persecutory, or delusions of control. Negative symptoms are based on emotional or behavioural deficits, and may include flattened affect, anhedonia, apathy, social withdrawal, and cognitive difficulties, such as decreased attention, concentration, learning, and memory. The annual incidence and overall prevalence of schizophrenia were recently estimated to be 0.2-0.4 per 1000 and 1% respectively (Jablensky 1997).

Both genetic factors and environmental factors may play a role in the development of schizophrenia. For example, at least seven genes are currently associated with schizophrenia (Harrison and Owen 2003; Harrison and Weinberger 2005). Pre- and perinatal events may also effect the development of schizophrenia, in addition to sociodemographic factors (for review, see Mueser and McGurk 2004).

Schizophrenia is currently treated, but not cured, using a variety of antipsychotic medications and psychosocial therapies. Despite the use of pharmacological therapies to treat schizophrenia for many years, the precise pathophysiology associated with this disease remains poorly understood. Diagnostic imaging is continually improving our understanding of structural changes related to schizophrenia. For example, studies using magnetic resonance imaging (MRI) have shown that schizophrenic patients generally show decreased regional brain volume and enlargement of the ventricular system in the brain (e.g., Van Horn and McManus 1992; Wright et al. 2000). Assessment of brain activity (using functional MRI - fMRI) or cerebral blood flow (using positron emission tomography - PET) has revealed that frontal areas of the brain may be dysfunctional in schizophrenic patients. For example, schizophrenics performing a cognitive task show decreased activity compared to control subjects in frontal and temporal areas (Yurgelun-Todd et al. 1996) and decreased blood flow in the frontal cortex (Weinberger et al. 1986). In addition, Okubo et al. (1997) have shown using PET that patients with schizophrenia demonstrate reduced D_1 DA receptors in the PFC that is related to severity of negative symptoms.

Despite advancements in diagnostic imaging, investigation into the neurochemistry of schizophrenia has yet to definitively identify the pathophysiology of this disorder. Recent work has explored the potential role of 5-hydroxytyptamine (5-HT or serotonin) in this disorder, at least partially due to the affinity of modern antipsychotic compounds for 5-HT receptor subtypes (see Meltzer et al. 2003). However, many studies have implicated, and continue to report, abnormal functioning of dopaminergic and glutamatergic systems in the brain; this is discussed further below.

The current DA hypothesis of schizophrenia, recently reviewed by Abi-Dargham (2004), has two basic elements: 1) excess subcortical DA is related to the positive symptoms of schizophrenia, and 2) a deficit of cortical DA is related to the negative and cognitive symptoms. Evidence for each of these components is based mainly on pharmacological manipulation of healthy volunteers or naïve animals. For example, chronic use of the indirect DA agonist amphetamine may induce psychosis (Connell 1958); similarly, repeated exposure to D_2 DA receptor agonists also induces positive symptoms (Einarson and Turchet 1983a,b). In addition, most clinically effective antipsychotic compounds demonstrate some ability to antagonize D_2 DA receptors (Creese et al. 1976; Seeman et al. 1976; Kapur and Seeman 2000). This decreased DA transmission may improve the perceptual abilities of patients with schizophrenia (Spohn et al. 1977). However, there is some evidence to suggest that the DA hypothesis of schizophrenia is not sufficient. For example, antipsychotics with affinity for D_2 DA receptors are often ineffective for the treatment of the negative symptoms of schizophrenia (Kinon and Lieberman 1996), and there is a substantial time difference between commencement of drug administration and onset of improvement in symptomatology (Marder and Van Putten 1995). In addition, DA metabolites and receptors may fall within normal range, both before and after treatment (Pickar et al. 1986; Farde et al. 1986). As such, it is generally accepted that DA function is related to, but not the only component of, the pathophysiology of schizophrenia. Research into the pathophysiology of schizophrenia has therefore been extended to include other neurotransmitters, such as Glu.

There is an abundance of literature that implicates Glu transmission in the neurochemistry of schizophrenia symptoms. For example, schizophrenic patients show decreased concentrations of Glu in cerebrospinal fluid (Kim et al. 1980). Phencyclidine (PCP; also called angel dust; crystal; killer weed etc.) is a Glu receptor antagonist that blocks the ion channel of the NMDA receptor complex. Like amphetamine, PCP may induce psychosis (Luby et al. 1959; Snyder 1980; Javitt and Zukin 1991). Similarly, other NMDA receptor antagonists such as ketamine demonstrate psychotomimetic effects in naïve human subjects (Johnson 1971). Several post-mortem radioligand binding studies have shown that NMDA receptors are altered in the frontal areas of schizophrenic patients (e.g., Toru et al. 1988; Deakin et al. 1989; Simpson et al. 1992), although confounding effects of antipsychotic medications before death on receptor expression in these patients are unclear. Taken together, altered NMDA receptor function is implicated in the pathophysiology of schizophrenia (Olney and Farber 1995).

Non-NMDA receptors may also be abnormal in schizophrenia. There are reports of decreased expression of AMPA receptor subunits in post-mortem brains from this patient population. Harrison et al. (1991) reported decreased GluR1 in the medial temporal lobe, while Eastwood et al. (1997) reported decreased GluR2/3 in the hippocampal regions of schizophrenic patients.

The DA and Glu hypotheses of schizophrenia are likely part of a much larger and complex mechanism; several authors have suggested that abnormal interactions *between* DA and Glu may underlie schizophrenia pathology. For example, decreased Glu input to the NAS may initiate psychosis (Carlsson and Carlsson 1990); this symptom may be concomitant with increased DA release (Grace 1992). A consequence may be reduced Glu transmission in the PFC that generates the negative symptoms (Toru et al. 1994).

General Methodology

There has been an abundance of animal studies aimed at exploring DA-Glu interactions in the context of reward and reinforcement. Before describing these animal studies further, it is necessary to clarify the terms reward and reinforcement. It is generally accepted that reward describes the phenomena whereby stimuli may elicit approach responses; in contrast, reinforcement describes how stimuli may strengthen, or reinforce, previously learned stimulus-response associations (White 1989). For the purposes of this thesis, and as found broadly in the literature, the terms reward and reinforcement are used interchangeably.

Animal studies of reward have mainly used four methods of measuring "motivated" behaviour in rodents: spontaneous locomotor activity, brain stimulation reward (BSR), place conditioning, and drug self-administration. The relevant DA-Glu interaction studies that have used these paradigms are described in Chapter 2. The former three were used in this thesis and are described in more detail below. While also of interest, the investigation of direct oral, intravenous, or intracranial self-administration of drugs, as recently described by Ikemoto and Wise (2004) and Ahmed and Koob (2005), was beyond the scope of this thesis.

Also beyond the scope of this thesis, but relevant to the investigation of DA-Glu interactions in reward, is the concept of behavioural sensitization. Briefly, repeated exposure to drugs of abuse may cause a progressive and long-term increase in the behavioural effects of the drug; this has been coined behavioural sensitization. Sensitized responses have been demonstrated in both human and animal studies that have used repeated administration of drugs of abuse, so sensitization is clearly an important part of the drug addiction literature. However, since the majority of experiments described in this thesis have used only acute administration of drugs, sensitization will not be discussed further.

Another behavioural paradigm related to motivation and schizophrenia is prepulse inhibition. In that paradigm, a sudden and intensive stimulus generates a startle reflex; exposure to a weak stimulus prior to the startle stimulus inhibits the startle reflex. Analogous effects are observed in rats and humans. This prepulse inhibition is decreased in schizophrenic patients, and this effect is reversed by antipsychotic medication (Swerdlow et al. 1994). However, since prepulse inhibition was not employed in this thesis, it too will not be discussed further.

In all of the studies described in this thesis, drugs were administered either systemically (intraperitoneally - IP or subcutaneously - SC) or by direct application into the NAS shell subregion ("by intracranial microinjection"). Prior to intracranial drug delivery, stainless steel cannulae were implanted stereotaxically and permanently fixed to the skull (see Yamamoto and Kutscher 1981; Greenshaw 1997a; Gardiner and Toth 1999). Following recovery, behavioural responses were assessed following microinjection of drug or vehicle solutions (0.5 μL). Though technically difficult, this method of drug delivery allows for the precise application of a drug to a very specific region. When combined with the use of pharmacologically selective compounds, this method may help to deduce the contribution of specific brain regions, neurotransmitters, or receptors to behaviour. While the less-precise administration of drugs systemically may be more representative of human methods of drug delivery, it is more difficult to account for nonspecific or peripheral effects of the parent compound and its metabolites (i.e., drug effects outside of the CNS). In addition, determination of drug effects in the brain following systemic administration may be difficult to detect if the drug does not easily permeate the blood-brain barrier.

Throughout this thesis, two dissimilar DA agonists were employed. 7-OH-DPAT (Mulder et al. 1987) (Figure 1.4) is currently the most selective D_3 DA agonist available. It binds preferentially to D_3 DA receptors, with >100, >1000, and >10,000-fold in vitro selectivity for D_3 over D_2, D_4 and D_1 DA receptors respectively (Lévesque et al. 1992) although in vivo affinities likely differ (Large and Stubbs 1994). Similarly, in vivo work shows that doses of 7-OH-DPAT lower than 0.12 μmol kg^{-1} (0.03 mg kg^{-1}) are mediated by D_3 DA receptors (Gainetdinov et al. 1995), and that low doses of 7-OH-DPAT (<0.1 mg kg^{-1}) do not interact with D_2 DA receptor binding sites as measured using EEDQ-induced D_2 DA receptor inactivation (Levant et al. 1996). In addition, Parsons et al. (1996) showed using reverse dialysis that 7-OH-DPAT dose-dependently decreased DA in the NAS, and this effect was blocked by coperfusion of the D_3 DA receptor antagonist nafadotride. Therefore, as suggested from in vitro studies, 7-OH-DPAT possesses significant D_3 DA receptor selectivity in vivo.

Behaviourally, low doses of 7-OH-DPAT induce hypoactivity that is blocked by the moderately selective D_3 DA receptor antagonist U99194A in mice (Gyertyán and Sághy 2004). In addition, Ireland et al. (2005) have shown that hypoactivity induced by low doses of another $D_{2/3}$ DA receptor agonist, quinelorane, increased neural activation in the NAS, an area rich in expression of D_3 DA receptors. These D_3 DA receptors may be located postsynaptically in the NAS, since the behavioural effects of low doses of 7-OH-DPAT may be independent of changes in DA release in the NAS (Waters et al. 1993; Svensson et al. 1994b,c; Mattingly et al. 1996; Depoortere et al. 1996; Zarandi 1998). However, there is also evidence that 7-OH-DPAT may stimulate a subpopulation of $D_{2/3}$ DA autoreceptors that influence DA synthesis and release in the NAS (Timmerman et al. 1991; Booth et al. 1994; Devoto et al. 1995; Pugsley et al. 1995). However, the discrepancy amongst studies is likely related to the dose-related effects of 7-OH-DPAT; as discussed throughout this thesis, numerous studies have suggested that higher doses of 7-OH-DPAT likely activate D_2 rather than D_3 DA receptors. It is therefore likely that pre- vs. postsynaptic effects of 7-OH-DPAT are dose-dependent; while low doses may selectively stimulate postsynaptic D_3 DA receptors, effects of intermediate doses are likely comprised of both pre- and postsynaptic activity (Zuch and Cory-Slechta 2001). The general pharmacology of D_3 DA receptors is further reviewed by Griffon et al. (1996).

Apomorphine (Figure 1.5) is a nonselective DA agonist that demonstrates equivalent inhibition of D_1-like (SCH 23390) and D_2-like (spiperone) radioligand binding in vitro; in contrast, apomorphine shows moderate $D_{2/3}$ DA receptor selectivity in vivo, as measured using raclopride receptor binding (Andersen and Jansen 1990). Behaviourally, low doses of apomorphine (<100 µg kg^{-1}) induce hypoactivity, an effect that has been widely attributed to stimulation of mesolimbic DA autoreceptors and associated with an inhibition of VTA cell firing and a decrease in DA release in the NAS in vivo (Strömbom 1975; Strömbom 1976; Aghajanian and Bunney 1977; Zetterström and Ungerstedt 1984; Yarbrough et al. 1984; Svensson et al. 1994b; Carey et al. 2004). These DA autoreceptors are presumably D_2-like DA receptors since behavioural effects of low doses of apomorphine are reversed by D_2-like DA receptor antagonists, but not D_1-like DA antagonists (Di Chiara et al. 1976; Strömbom 1977; Ståhle and Ungerstedt 1984; Gessa et al. 1985; Cuomo et al. 1986; Ståhle and Ungerstedt 1986; Radhakishun and Van Ree 1987; Imperato et al. 1988).

Taken together, comparative effects of apomorphine and 7-OH-DPAT may contribute to the differentiation of pre- vs. postsynaptic interactions within the mesolimbic system.

Figure 1.4. Structure of 7-OH-DPAT

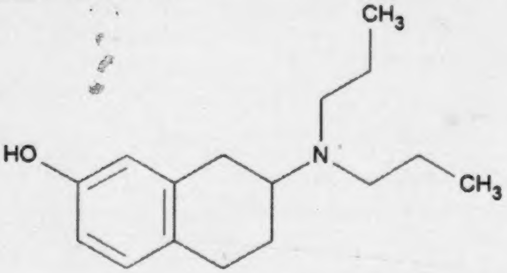
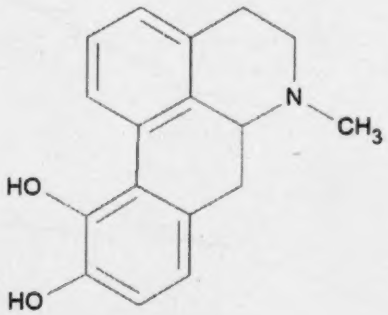


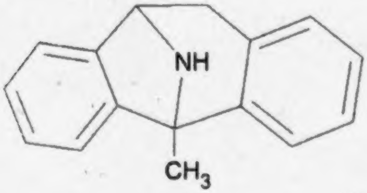
Figure 1.5. Structure of apomorphine



In addition to these DA receptor agonists, this thesis also employed Glu receptor antagonists of the NMDA and AMPA/kainate receptor subtypes. NMDA receptors are widely distributed in the mammalian CNS (Cooper et al. 2003). Of particular interest in the present work is the expression of NMDA receptors in the terminal region (NAS) of dopaminergic neurons originating in the VTA

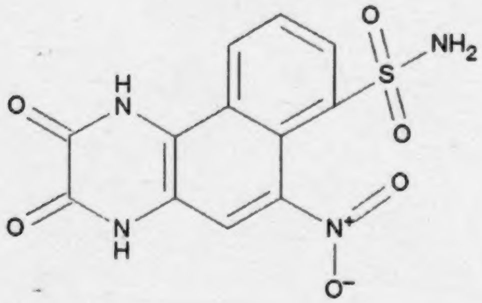
(Monaghan and Cotman 1985). Intravenous administration of noncompetitive (PCP or MK-801) or competitive (CGP 39551) NMDA receptor antagonists may elevate extracellular DA in the NAS shell (Marcus et al. 2001). With respect to the experiments described in this thesis, we have employed the well-studied NMDA receptor antagonist MK-801 (Figure 1.6).

Figure 1.6. Structure of MK-801.



AMPA receptors are also widely distributed in the mammalian CNS (Cooper et al. 2003). Like NMDA receptors, AMPA receptors have been localized to the NAS (Cotman and Iversen 1987; Bettler and Mulle 1995) and ample evidence indicates a role for AMPA receptors in the NAS shell in the neural mechanisms of reward and reinforcement. For example, administration of AMPA receptor antagonists into this area has been shown to reverse increases in locomotor activity or conditioned place preferences induced by psychostimulants such as amphetamine or cocaine (see Chapter 2). As such, the most selective AMPA/kainate receptor antagonist available (NBQX) was also employed in this thesis (Figure 1.7.).

Figure 1.7. Structure of NBQX.



As described above, this thesis used three methods of measuring animal behaviour related to motivation and reinforcement. While the measurement of spontaneous locomotor activity is considered a general measure of arousal, BSR elicits a change in the excitatory state of a targeted brain region in response to lever-pressing (primary reinforcement), while place preference or aversion testing assesses drug-induced conditioning (secondary reinforcement). Each of these measurements will be explained further below.

Spontaneous locomotor activity

This thesis has investigated both the acute and conditioned effects of drugs on spontaneous locomotor activity. Spontaneous locomotor activity is widely used as a gross index of the stimulant or depressant properties of drugs. The animals' exploration about the environment is a complex behaviour affected by sensory processing, environmental cues, motor responses, and the animals' state of arousal (Kelley et al. 1989).

In our laboratory, spontaneous locomotor activity is monitored using several Plexiglas chambers, each equipped with a grid of 36 infrared beams. Briefly, the interruption of beams that are projected from a light source on one side of the box to a light-activated switch on the opposite side are computer-recorded to account for horizontal activity, rearing, and consecutive (stereotypic) behaviours. Clearly a limitation of this method of recording spontaneous exploration is the lack of differentiation between walking, circling, grooming, scratching, and head or tail movements.

Throughout each locomotor activity experiment, the following measurements were computer-recorded during each session:

- Horizontal activity (total number of lower beam breaks).
- Consecutive activity (repetitive breaking of one beam or stereotypy).
- Vertical activity (number of upper beam breaks or rearing).

Brain stimulation reward

In the paradigm of brain stimulation reward (BSR) or intra-cranial self-stimulation (ICSS), animals learn to press a lever, or perform some other task, when such responses are reinforced by electrical stimulation of specific brain regions (Olds and Milner 1954). This "habit-forming" behaviour is analogous to behaviours performed for natural rewards (such as food or sexual contact) (Wise

1996). This paradigm has been used to determine the brain regions that are potentially reinforcing or aversive, and to assess reward-increasing or reward-decreasing effects of drugs.

Studies of BSR must always be interpreted with caution, as measures may be based simply on response rate. The nonspecific stimulant or sedative effects of drugs per se, or drug-induced changes is attention or memory, may affect this measure (Iversen 1977). Other important factors to consider are the parameters of electrical current, frequency, pulse length, and train length. A preferred method of measuring BSR is to use "threshold" measures of reinforcement that consist of stepwise changes in either electrical current or frequency (see Miliaressis et al. 1986; Konkle et al. 2001). Threshold measures are considered more specific and sensitive to drug-induced changes in reward (rather than nonspecific performance effects). The threshold measure used in the present study is referred to as rate-frequency threshold analysis; the electrical frequency that maintains 50% of the maximal response rate is used as an indicator of reward. Changes in frequency were employed rather than changes in current; as the radius of the neuronal area stimulated may vary with changes in current, alteration of current intensity may alter the cell population being stimulated, increasing variability of results (Gallistel et al. 1981). Altering stimulation frequency minimizes this variability. More methodological detail is provided in the following chapters that have employed measurement of BSR.

As described above, the mesolimbic DA system is associated with the rewarding and addictive properties of drugs abused by humans, such as amphetamine, cocaine, and nicotine (White 1996; Koob and Le Moal 1997). Interestingly, these compounds generally facilitate BSR (for review, see Wise 1996), and BSR is associated with changes in DA output in the NAS (Fibiger et al. 1987). There is also evidence to suggest that Glu may affect BSR. Briefly, NMDA receptor antagonists, when given systemically or directly into the NAS, decrease reward thresholds (e.g., Carlezon and Wise 1996), while AMPA receptor ligands appear to have little effect on BSR when given alone (Choi 2000).

In the BSR paradigm, several computer programs were used to train animals and assess baseline performance (this is described further in subsequent chapters). For tests of drug effects, the following measurements were computer-recorded:

- Rate-frequency threshold (M_{50}): frequency for 50% maximum response.
- Maximum response (RMAX): total lever-presses at one frequency.
- Total responses (TRES): total lever-presses for an entire session.

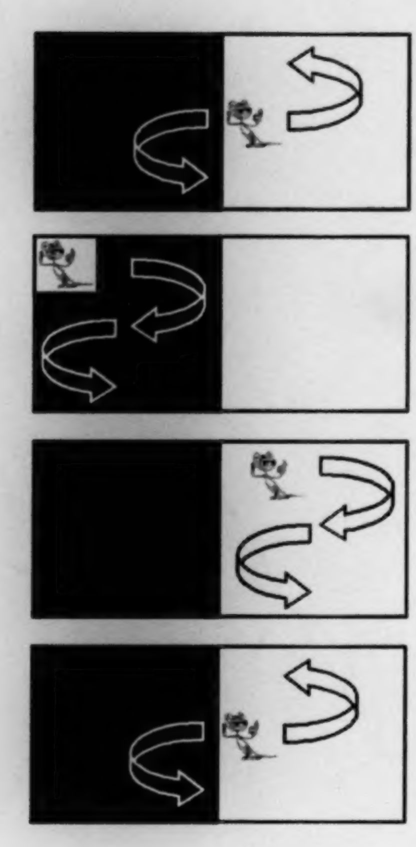
Place conditioning

Place conditioning, as reviewed by several authors (Hoffman 1989; Tzschentke 1998; Carlezon 2003), is widely used as a method for measuring the conditioned reinforcing effects of a stimulus, including pharmacological compounds, food, or availability of a sexual partner. Briefly, this paradigm employs "secondary conditioning" by pairing a neutral stimulus (i.e., floor texture) with a rewarding or aversive stimulus (i.e., drug) to establish a conditioned place preference (CPP) or aversion (CPA).

In the experiments described in this thesis, animals were exposed to two distinct neutral environments that differed in floor texture; time spent in each was recorded as a measure of baseline preference. Then, one environment was repeatedly paired with drug administration and the other with vehicle administration. Later, in the absence of drug administration, animals were given free access to both environments; again, time spent in each was recorded. The time spent in the drug-paired side, relative to baseline preference, was used as a measure of conditioned reinforcement (i.e., an increase in time spent in the drug-paired environment indicated a CPP). This procedure is illustrated in Figure 1.8.

According to Swerdlow et al. (1989), three processes are necessary to establish place conditioning: 1) the drug must induce a change in the internal affective state of the animal; 2) the animal must form an association between the change in affect and the neutral stimulus; and 3) this association must be recalled and used to direct approach or avoidance responses.

Figure 1.8. Schematic of the place conditioning procedure (Adapted from Biondo 2002).



Pre-conditioning (free access)

Conditioning (restricted to one side)

Conditioning (restricted to other side)

Retention testing (free access)

The contribution of locomotor responses to the establishment and retention of place conditioning is controversial. While psychostimulants may induce robust hyperactivity and CPP, there is evidence to suggest that exploration about the environment is not necessary for the establishment of conditioned responses. For example, if animals are confined and cannot explore the environment during conditioning, they may still demonstrate conditioned locomotor responses to amphetamine, indicating the formation of a drug-environment association in the absence of a CPP or CPA (Carr et al. 1988; Swerdlow et al. 1989). Similarly, if animals are sedated and cannot explore the environment during conditioning, they may still demonstrate conditioned place preferences. For example, the GABA receptor ligand zolpidem induces sedation but fails to disrupt CPP induced by cocaine or amphetamine (Meririnne et al. 1999). These studies suggest that exploration of the environment (or lack thereof) during drug conditioning may be independent of conditioned drug effects. Therefore, to complement the place conditioning studies described in this thesis, we have also explored the contribution of conditioned locomotor effects to drug-induced place preferences (see Chapter 6).

The next chapter will review the available literature that has investigated DA-Glu interactions in the context of motivated behaviour in laboratory animals. The objectives of this thesis are described at the end of Chapter 2.

Chapter 2. A review of dopamine and glutamate in reward

Introduction

In the mammalian brain, excitatory amino acids such as Glu may regulate motivation and reward by influences on mesolimbic DA. The interaction between DA and Glu in this context is significant because, as described in Chapter 1, the brain areas associated with motivation primarily communicate by DA and Glu signals. The purpose of this review is to summarize the available literature that has investigated interactions between DA receptor-related and Glu receptor-related compounds on motivated behaviours. Specifically, in relation to the experiments described in subsequent chapters, this review is limited to studies that have used Glu receptor antagonists and direct or indirect DA agonists.

The relevant studies are reviewed below according to measurements of 1) spontaneous locomotor activity; 2) brain stimulation reward (BSR); 3) drug self-administration; and 4) place conditioning. While drug self-administration was not directly employed in this thesis, studies using this paradigm have contributed significantly to our understanding of DA-Glu interactions in reward-related processes. When possible, the studies are categorized according to the ligands used to induce blockade of NMDA or non-NMDA Glu receptors. Within each section, an important distinction may also be made between competitive and noncompetitive antagonists. Whereas competitive antagonists may bind to the same site as Glu, noncompetitive antagonists may bind to a different site of the receptor complex to induce inactivation. Finally, this chapter concludes with a brief summary of the trends (and gaps) observed in the literature across the four reward-related behaviours, and a statement of the thesis objectives.

Locomotor activity

Numerous studies have examined the interaction between NMDA receptor antagonists, such as AP5 (also called APV), and psychostimulants. For example, Pulvirenti et al. (1991) reported that locomotor activation induced by cocaine (10 mg kg^{-1}) was attenuated by intra-NAS APV (3 μg). Kelley and Throne (1992) further localized a possible site of this effect by demonstrating that intra-NAS AP5 (0.5 or 1.0 μg) dose-dependently reduced hyperactivity induced by intra-NAS amphetamine (5.0 μg). Subsequently, Burns et al. (1994) replicated this result by demonstrating that intra-NAS AP5 (0.59 μg) completely blocked the effect of intra-NAS amphetamine (3 μg). The inhibitory effects of AP5 on amphetamine-induced hyperactivity are surprising since AP5 alone increased activity in both studies. However, one study that has investigated the interaction between AP5 and both amphetamine and methamphetamine did not demonstrate hyperactivity induced by AP5 alone. In that study, Shoblock et al. (2003) showed that intra-NAS shell AP5 (0.5 μg) reduced hyperactivity due to amphetamine (2 mg kg^{-1}) but not methamphetamine (2 mg kg^{-1}). David et al. (2004) also showed that intra-NAS core amphetamine (5 μg) induced hyperactivity that was reduced, but not blocked, by D-AP5 (0.1 μg).

Using more selective DA agonists, David et al. (2004) reported that intra-NAS core microinjection of D-AP5 (0.1 μg) was ineffective alone but blocked the stimulatory effect of intra-NAS core D_1 DA agonist SKF 38393 (1 μg). In addition, intra-NAS core $D_{2/3}$ DA agonist quinpirole (1 μg) decreased activity, and this effect was potentiated by D-AP5 (0.1 μg). When given in combination, SKF 38393 + quinpirole (1 μg each) induced hyperactivity that was blocked by D-AP5 (0.1 μg). The authors suggested that there may be synergistic activity between NMDA receptors and D_1-like DA receptors, and an antagonistic action between NMDA receptors and D_2-like DA receptors.

In contrast to the hyperactivity induced by psychostimulants, Dall'Olio et al. (1997) investigated the effects of NMDA antagonists on *hypoactivity* induced by D_2-like DA agonists 7-OH-DPAT (0.005-0.08 mg kg^{-1}) or quinpirole (0.0125-0.050 mg kg^{-1}). In that study, the noncompetitive NMDA antagonist MK-801 (0.06 mg kg^{-1}) was ineffective alone and prevented the decrease in activity induced by 7-OH-DPAT, but not quinpirole. Dall'Olio et al. (1997) suggested that MK-801 interacts with D_3 DA receptor activity coupled to locomotor hypoactivity; the lack of interaction with quinpirole is likely related to its ability to reduce activity through the stimulation of presynaptic D_2 DA autoreceptors (Dall'Olio et al. 1997).

The interaction between MK-801 and subtype-specific DA receptor stimulation was investigated further by Leriche et al. (2003) using the D_3 DA partial agonist BP 897. In that study, BP 897 (0.3 or 1.0 mg kg^{-1}) alone was ineffective but dose-dependently reduced hyperactivity induced by MK-801 (0.12 mg kg^{-1}). It is noteworthy that BP 897 is a partial agonist in vitro and acts in vivo either as an agonist when DA is depleted or an antagonist when there is sustained receptor stimulation by DA (Pilla et al. 1999), as occurs following administration of noncompetitive NDMA receptor antagonists such as MK-801.

In addition to the studies described above using AP5, two other studies have examined the effects of competitive NMDA antagonists on changes in locomotor activity induced by DA receptor stimulation. Witkin (1993) reported that NPC 12626 (30 mg kg^{-1}) decreased hyperactivity induced by cocaine (30 mg kg^{-1}) or methamphetamine (3 mg kg^{-1}). In contrast to the effects described above using MK-801, competitive Glu antagonists may be ineffective on *hypoactivity* induced by D_2-like DA agonists. As described above, Dall'Olio et al. (1997) used 7-OH-DPAT (0.005-0.08 mg kg^{-1}) or quinpirole (0.0125-0.050 mg kg^{-1}) to induce hypoactivity; in contrast to MK-801, CGP 43487 (0.75 mg kg^{-1}) was ineffective alone and did not affect the responses to 7-OH-DPAT nor quinpirole.

Taken together, these studies suggest that competitive NMDA antagonists may interrupt the stimulatory effect of psychostimulants; the effects of noncompetitive NMDA receptor antagonists on hyperactivity induced by psychostimulants are unknown. With respect to *hypoactivity* induced by low doses of $D_{2/3}$ DA agonists, noncompetitive antagonism, but not competitive, may disrupt this inhibitory effect.

There are several reports that have investigated the role of the modulatory glycine site of the NMDA receptor complex in locomotor activity. Hutson et al. (1991) reported that administration of an antagonist of this site, (+)-HA-966, at 30 mg kg^{-1}, attenuated but did not block hyperactivity induced by systemic amphetamine (0.75 mg kg^{-1}). However, when amphetamine was given directly into the NAS (10 μg), (+)-HA-966 (30 mg kg^{-1}) completely prevented the stimulant effect of amphetamine (Hutson et al. 1991). (+)-HA-966 (10 or 30 mg kg^{-1}) alone was not effective. In addition, it has also been shown that (+)-HA-966 (100 mg kg^{-1}) or another glycine site antagonist, 7-chlorokynurenic acid (100 or 300 mg kg^{-1}), blocked the locomotor stimulant effect of methamphetamine (3 mg kg^{-1}), but not cocaine (30 mg kg^{-1}) (Witkin 1993). It is noteworthy that the antagonists were ineffective when given alone, with the exception of (+)-HA-966 that decreased activity at 100 mg kg^{-1}. These studies suggest that blockade of NMDA receptor function via the modulatory glycine site may also interrupt hyperactivity induced by psychostimulants.

The role of non-NMDA Glu receptors in this context has been widely investigated. In fact, the first report of an interaction between a Glu antagonist and DA agonist used the nonselective non-NMDA antagonist glutamate diethyl ester (GDEE). Pulvirenti et al. (1989) reported that intra-NAS administration of GDEE (10, 20 μg) attenuated hyperactivity induced by cocaine (20 mg kg^{-1}). In addition, GDEE (20 μg) reduced hyperactivity induced by a lower dose of cocaine (10 mg kg^{-1}) or by amphetamine (0.75 mg kg^{-1}). While the effects of GDEE alone were not reported, Pulvirenti et al. (1989) suggested that Glu input to the NAS might be important for the generation of hyperactivity following the administration of psychostimulants.

Subsequent studies employed other AMPA/kainate receptor antagonists. Willins et al. (1992) showed that intra-NAS administration of the AMPA receptor antagonist DNQX (1.0 μg) inhibited hyperlocomotion induced by amphetamine (0.5 mg kg^{-1}) by 55%, while DNQX alone was ineffective. In addition, microinjection of DNQX (1.0 μg) into the ventral pallidal region reduced amphetamine hyperactivity by 78% without altering activity when given alone. Kaddis et al. (1993) also showed that intra-NAS DNQX (1.0 μg) was ineffective alone, and that it inhibited the locomotor stimulation induced by systemic or intra-NAS cocaine (20 mg kg^{-1} or 20 μg). Using more selective DA agonists, Kaddis et al. (1993) showed that intra-NAS DNQX inhibited the hyperactivity induced by intra-NAS administration of a combination of SKF 38393 (3 μg) and quinpirole (0.3 μg). However, there is contradictory evidence for the effects of DNQX. For example, intra-NAS shell DNQX (0.25 μg) was ineffective alone and did not significantly change the stimulant response of 2 mg kg^{-1} of amphetamine or methamphetamine (Shoblock et al. 2003). This discrepancy may be dose-related as the dose of DNQX used was lower than that previously used by Willins et al. (1992) to decrease amphetamine-induced hyperactivity (0.25 vs. 1.0 μg respectively).

Behavioural effects of the selective competitive AMPA/kainate receptor antagonists CNQX and NBQX have also been investigated. For example, Burns et al. (1994) reported that intra-NAS CNQX (0.48 µg) completely blocked the stimulatory effect of intra-NAS amphetamine (3.0 µg); this is surprising since CNQX alone increased activity. NBQX (3-30 mg kg^{-1}) has also been shown to dose-dependently decrease hyperactivity induced by cocaine (30 mg kg^{-1}) or methamphetamine (3 mg kg^{-1}) (Witkin 1993). Vanover (1998) also showed that NBQX (80 mg kg^{-1}) attenuated amphetamine hyperactivity (3.0 mg kg^{-1}). It is noteworthy that NBQX, when given alone, dose-dependently decreased spontaneous activity in that study (Vanover 1998).

Previous work from this laboratory has investigated the interaction between CNQX or NBQX and $D_{2/3}$ DA agonists on spontaneous locomotor activity. In those studies (Choi 2000, Choi et al. 2000), intra-NAS CNQX (0.5 µg) alone was ineffective but potentiated a decrease in activity induced by intra-NAS 7-OH-DPAT (5.0 µg) whether compounds were given into the NAS shell or core subregion. In addition, similar potentiation was observed when an ineffective dose of NBQX (0.5 µg) was given in combination with intra-NAS shell 7-OH-DPAT. In contrast, the stimulant effects of SKF 38393 (5.0 µg) were unaltered by CNQX (0.5 µg) in the NAS shell or core subregions. These studies suggested that $D_{2/3}$ DA receptor stimulation and AMPA/kainate receptor blockade may act synergistically in the NAS to alter spontaneous exploration. In agreement with these findings, David et al. (2004) recently reported that intra-NAS core quinpirole (1 μg) decreased activity, and this effect was potentiated by CNQX (0.3 μg). However, in contrast to the results described above, intra-NAS core CNQX (0.3 μg) was ineffective alone but blocked the stimulatory effect of intra-NAS core SKF 38393 (1 μg). The reason for this discrepancy is not clear. In addition to those results, David et al. (2004) also showed that intra-NAS core amphetamine (5 μg) induced hyperactivity that was reduced, but not blocked, by CNQX (0.3 μg), while a combination of SKF 38393 + quinpirole (1 μg each) induced hyperactivity that was reduced by CNQX (0.3 μg).

Vanover (1998) investigated the effects of another competitive AMPA receptor antagonist, LY 326325, on behavioural stimulation induced by amphetamine (3.0 mg kg^{-1}). In that study, LY 326325 (80 mg kg^{-1}) attenuated amphetamine-induced hyperactivity, and dose-dependently decreased activity when given alone.

Taken together, studies that have used competitive AMPA/kainate receptor antagonists suggest that activation of AMPA/kainate receptors in the NAS may be necessary for the hyperactivity induced by psychostimulants. In addition, competitive AMPA/kainate antagonists may potentiate *hypoactivity* induced by some DA agonists.

Two studies have employed the noncompetitive AMPA receptor antagonist GYKI 52466. Maj et al. (1995b) studied the effects of this ligand on hyperactivity induced by cocaine (20 mg kg^{-1} in mice) or apomorphine (0.3 mg kg^{-1} in rats). In mice, GYKI 52466 (1, 3, or 10 mg kg^{-1}) alone decreased activity while only the highest dose increased cocaine-induced hyperactivity. In rats, GYKI 52466 (10 mg kg^{-1}) alone decreased locomotor activity while lower doses were ineffective. When combined, GYKI 52466 (3 mg kg^{-1}) increased apomorphine-induced hyperactivity. Vanover (1998) also showed that GYKI 52466 (5-20 mg kg^{-1}) and another noncompetitive AMPA antagonist LY 300164 (3-5.6 mg kg^{-1}) attenuated hyperactivity induced by amphetamine (3.0 mg kg^{-1}). It is noteworthy that Vanover (1998) reported that the AMPA receptor antagonists, when given alone, dose-dependently decreased spontaneous activity. More studies using noncompetitive AMPA antagonists are needed to clarify the effects of such antagonists on psychostimulant-induced hyperactivity.

There are also a number of studies that have investigated interactions between Glu receptor antagonists and DA agonists using DA-depleted animals (e.g., Svensson et al. 1994a; Starr and Starr 1995; Gossel et al. 1995). However, the widespread effects of global DA depletion are beyond the scope of this thesis, since the experiments described in the following chapters have used only DA-intact animals.

A tabulation of the effects of Glu receptor antagonists and DA agonists on locomotor activity is shown in Table 2.1 (systemic DA agonists) and Table 2.2 (centrally administered DA agonists). The main findings from systemic studies can be summarized as follows: 1) amphetamine-induced hyperactivity is attenuated by Glu receptor blockade; 2) cocaine-induced hyperactivity is attenuated by Glu receptor blockade, but may be blocked by intra-NAS

AMPA/kainate antagonists; 3) methamphetamine-induced hyperactivity may be blocked by glycine site antagonists; and 4) MK-801 may block effects of selective D_3 DA receptor stimulation. The main findings from central studies can be summarized as follows: 1) effects of Glu receptor antagonists on hyperactivity induced by intra-NAS amphetamine are unclear; 2) effects of intra-NAS D_1 DA receptor stimulation may be blocked by intra-NAS Glu receptor blockade; and 3) intra-NAS effects of 7-OH-DPAT are potentiated by AMPA/kainate receptor blockade.

Table 2.1. Review of locomotor activity studies that have investigated effects of systemically administered DA agonists and Glu antagonists. NE = no effect, NR = not reported. Arrows represent the direction of change of locomotor activity.

DA Agonist (Effect)	Glu Antagonist (Effect)	Combined Effect	Reference
Amphetamine (↑)	GDEE (NR)	Attenuated	Pulvirenti et al. 1989
	Intra-NAS shell AP5 (NE)	Attenuated	Shoblock et al. 2003
	(+)-HA-966 (NE)	Attenuated	Hutson et al. 1991
	Intra-NAS DNQX (NE)	Attenuated	Willins et al. 1992
	Intra-NAS shell DNQX (NE)	NE	Shoblock et al. 2003
	Intra-VP DNQX (NE)	Attenuated	Willins et al. 1992
	NBQX (↓)	Attenuated	Vanover (1998)
	GYKI 52466 (↓)	Attenuated	Vanover (1998)
	LY 300164 (↓)	Attenuated	Vanover (1998)
	LY 326325 (↓)	Attenuated	Vanover (1998)
Cocaine (↑)	GDEE (NR)	Attenuated	Pulvirenti et al. 1989
	Intra-NAS APV (NR)	Attenuated	Pulvirenti et al. 1991
	NPC 12626 (NE)	Attenuated	Witkin 1993
	(+)-HA-966 (↓)	NE	Witkin 1993
	7-CKA (NE)	NE	Witkin 1993
	Intra-NAS DNQX (NE)	Blocked	Kaddis et al. 1993
	NBQX (NE)	Attenuated	Witkin 1993
	GYKI 52466 (↓)	Increased	Maj et al. 1995b
Methamphetamine (↑)	Intra-NAS shell AP5 (NE)	NE	Shoblock et al. 2003
	NPC 12626 (NE)	Attenuated	Witkin 1993
	(+)-HA-966 (↓)	Blocked	Witkin 1993
	7-CKA (NE)	Blocked	Witkin 1993
	Intra-NAS shell DNQX (NE)	NE	Shoblock et al. 2003
	NBQX (NE)	Attenuated	Witkin 1993
Apomorphine (↑)	GYKI 52466 (NE)	Increased	Maj et al. 1995b
7-OH-DPAT (↓)	MK-801 (NE)	Blocked	Dall'Olio et al. 1997
	CGP 43487 (NE)	NE	Dall'Olio et al. 1997
Quinpirole (↓)	MK-801 (NE)	NE	Dall'Olio et al. 1997
	CGP 43487 (NE)	NE	Dall'Olio et al. 1997
BP 897 (NE)	MK-801 (↑)	Blocked	Leriche et al. 2003

Table 2.2. Review of locomotor activity studies that have investigated effects of centrally administered DA agonists and Glu antagonists. NE = no effect, NR = not reported. Arrows represent the direction of change of locomotor activity.

DA Agonist (Effect)	Glu Antagonist (Effect)	Combined Effect	Reference
Intra-NAS Amphetamine (↑)	AP5 (↑)	Blocked	Burns et al. 1994
	Intra-NAS AP5 (NE)	Attenuated	Kelley and Throne 1992
	(+)-HA-966 (NE)	Blocked	Hutson et al. 1991
	CNQX (↑)	Blocked	Burns et al. 1994
Intra-NAS core Amphetamine (↑)	Intra-NAS core D-AP5 (NE)	Attenuated	David et al. 2004
	Intra-NAS core CNQX (NE)	Attenuated	David et al. 2004
Intra-NAS cocaine (↑)	Intra-NAS DNQX (NE)	Blocked	Kaddis et al. 1993
Intra-NAS shell SKF 38393 (↑)	Intra-NAS shell CNQX (NE)	NE	Choi et al. 2000
Intra-NAS core SKF 38393 (↑)	Intra-NAS core D-AP5 (NE)	Blocked	David et al. 2004
	Intra-NAS core CNQX (NE)	NE	Choi et al. 2000
	Intra-NAS core CNQX (NE)	Blocked	David et al. 2004
Intra-NAS core Quinpirole (↓)	Intra-NAS core D-AP5 (NE)	Potentiated	David et al. 2004
	Intra-NAS core CNQX (NE)	Potentiated	David et al. 2004
Intra-NAS SKF 38393 + Quinpirole (↑)	Intra-NAS DNQX (NE)	Blocked	Kaddis et al. 1993
Intra-NAS core SKF 38393 + Quinpirole (↑)	Intra-NAS core D-AP5 (NE)	Blocked	David et al. 2004
	Intra-NAS core CNQX (NE)	Attenuated	David et al. 2004
Intra-NAS shell 7-OH-DPAT (↓)	Intra-NAS shell CNQX (NE)	Potentiated	Choi et al. 2000
	Intra-NAS core NBQX (NE)	Potentiated	Choi et al. 2000
Intra-NAS core 7-OH-DPAT (↓)	Intra-NAS shell CNQX (NE)	Potentiated	Choi et al. 2000

Brain stimulation reward

Studies of effects of Glu receptor antagonists and DA agonists on BSR are less common in the literature than studies of locomotor activity. To the best of our knowledge, the results of only a few such BSR studies are available.

Two studies have reported interactions between NMDA receptor-related ligands and cocaine-induced potentiation of BSR. Ranaldi et al. (1997) reported that MK-801 (0.05 mg kg^{-1}) or cocaine (4 mg kg^{-1}) alone did not affect rate-frequency threshold measures of lateral hypothalamus (LH) BSR. When combined, these doses of MK-801 and cocaine reduced rate-frequency thresholds (i.e., increased reward). The authors concluded that NMDA receptor blockade by MK-801 enhanced the rewarding properties of cocaine. Subsequently, Tzschentke and Schmidt (2000) investigated the effects of another noncompetitive NMDA receptor antagonist, memantine, on cocaine-induced potentiation of MFB BSR. That study showed that cocaine (5 mg kg^{-1}) decreased rate-frequency thresholds, and this reward-increasing effect was not changed by memantine (5 mg kg^{-1}). In contrast, a higher dose of cocaine (10 mg kg^{-1}) also decreased rate-frequency thresholds, and this effect was further enhanced by a higher dose of memantine (10 mg kg^{-1}). It is noteworthy that memantine alone was ineffective. Similar to Ranaldi et al. (1997), Tzschentke and Schmidt (2000) concluded that NMDA receptor blockade may add to DA stimulation induced by cocaine, or alternatively, could reduce the activity of medium spiny GABAergic neurons in the NAS to act in synergy with the DA-related effects of cocaine.

Bespalov and Zvartau (1995) were the first to report an interaction between AMPA/kainate receptor blockade and amphetamine; however this study was done in the context of sensitization to morphine. More specifically, Bespalov and Zvartau (1995) showed that administration of morphine for one week sensitized animals to a challenge of amphetamine (1.5 mg kg^{-1}) using rate-current threshold measurements of MFB BSR. Coadministration of DNQX (100 mg kg^{-1}) with morphine prevented the sensitization to amphetamine challenge, without affecting BSR when given alone. The authors concluded that lasting changes in sensitivity to drugs, induced by repeated administration, is associated with non-NMDA Glu receptor mechanisms.

Previous work from this laboratory (Choi 2000) has investigated the interaction between AMPA/kainate receptor blockade and DA receptor stimulation in the context of VTA BSR. Specifically, Choi (2000) showed that neither CNQX (0.5 μg) nor 7-OH-DPAT (5.0 μg) significantly affected rate-frequency thresholds when microinjected into the NAS shell or core subregions. However, these drugs combined increased rate-frequency thresholds (i.e., decrease reward) when injected into the NAS shell or core subregions. Choi (2000) suggested that concomitant blockade of AMPA/kainate receptors and stimulation of $D_{2/3}$ DA receptors in the NAS may have synergistic reward-decreasing effects.

A tabulation of the effects of Glu receptor antagonists and DA agonists on brain stimulation reward is shown in Table 2.3. The main findings from this section of the review can be summarized as follows: 1) cocaine decreases BSR rate-frequency thresholds, indicating increased reward, and this effect is potentiated by memantine; 2) lower doses of cocaine may be ineffective, but when combined with MK-801, but not memantine, increase reward, and 3) in the NAS shell, a combination of 7-OH-DPAT and AMPA/kainate receptor blockade increases rate-frequency thresholds, or decreases reward.

Table 2.3. Review of brain stimulation reward studies that have investigated effects of DA agonists and Glu antagonists. NE = no effect, NR = not reported. Arrows represent the direction of change of rate-frequency thresholds.

DA Agonist (Effect)	**Glu Antagonist (Effect)**	**Combined Effect**	**Reference**
Cocaine 4 mg kg^{-1} (NE)	MK-801 0.05 mg kg^{-1} (NE)	↓	Ranaldi et al. 1997
Cocaine 5 mg kg^{-1} (↓)	Memantine 5 mg kg^{-1} (NE)	No change	Tzschentke and Schmidt 2000
Cocaine 10 mg kg^{-1} (↓)	Memantine 10 mg kg^{-1} (NE)	Potentiated ↓	Tzschentke and Schmidt 2000
Intra-NAS shell 7-OH-DPAT (NE)	Intra-NAS shell CNQX (NE)	↑	Choi 2000
Intra-NAS core 7-OH-DPAT (NE)	Intra-NAS core CNQX (NE)	↑	Choi 2000

Drug self-administration

Similar to studies of BSR, reports of effects of Glu receptor antagonists and DA agonists in drug self-administration are also less common in the literature than studies of locomotor activity. Surprisingly, Glu antagonists have only been investigated in combination with the self-administration of cocaine; no studies have yet employed the self-administration of amphetamine or more selective DA agonists.

The first study to report an interaction between Glu receptor blockade and DA receptor stimulation in this context used self-administration of cocaine (0.75 mg kg^{-1} per IV injection) in combination with a competitive NMDA receptor antagonist. In that study, intra-NAS APV (1.5 or 3.0 µg) caused a dose-dependent increase in responding for cocaine (Pulvirenti et al. 1992). The effect of intra-NAS NMDA receptor blockade was also investigated by Cornish et al. (1999). In that study, rats were trained to self-administer cocaine (1.0 mg kg-1 per IV injection), and following training, received intra-NAS AP5 prior to the self-administration session. In contrast to the above result, intra-NAS AP5 (1.97 µg) did not affect responding for the active lever.

The majority of studies targeting NMDA receptor inactivation in the context of drug self-administration have used the noncompetitive antagonist MK-801. Schenk et al. (1993a) showed using experienced cocaine self-administering rats that acute administration of MK-801 prior to the test session did not reliably shift the dose-response curve for cocaine. Specifically, MK-801 (0.25 mg kg^{-1})

suppressed responding for low doses of cocaine (0.06-0.25 mg kg^{-1} per IV injection), but was ineffective at altering responding for higher doses (0.5-2.0 mg kg^{-1} per IV injection). A noteworthy finding was that MK-801 induced a complete loss of discriminative responding for cocaine (i.e., inactive lever responses were increased), that suggests MK-801 disrupted the operant self-administration procedure (Schenk et al. 1993a). Similarly, Cornish et al. (1999) showed using animals self-administering cocaine (1.0 mg kg^{-1} per IV injection) that intra-NAS MK-801 (0.38 µg or 3.8 µg) did not affect responding for the active lever, although MK-801 (0.38 µg) increased responding for the inactive lever. However, this potential nonspecific effect of MK-801 has not been consistently reported. For example, Shoaib et al. (1995) also investigated effects of MK-801 on self-administration of cocaine (0.33 mg kg^{-1} per IV injection). In that study, MK-801 (0.3 mg kg^{-1}) but not lower doses (0.03 or 0.1 mg kg^{-1}) suppressed cocaine self-administration without affecting operant responding for food reward.

The effects of MK-801 on cocaine self-administration were investigated further by Ranaldi et al. (1996) using measurements of breaking points under a progressive ratio schedule of reinforcement. Under this schedule, the ratio of responses required to obtain the next injection progressively increased from 1 to 603; the breaking point was defined as the last completed increment in the schedule to obtain cocaine. Using a range of cocaine doses (0.19-1.50 mg kg^{-1} per IV injection), Ranaldi et al. (1996) showed that lower doses of cocaine were associated with lower breaking points, while higher doses were associated with higher breaking points. Generally, MK-801 (0.05-0.15 mg kg^{-1}) increased breaking points, whereas MK-801 (0.2 mg kg^{-1}) decreased that measure. Ranaldi et al. (1996) concluded that the effect of MK-801 on cocaine breaking points fitted an inverted-U function, suggesting that MK-801 increased the rewarding efficacy of cocaine. This would agree with the findings of Shoaib et al. (1995) but contrasts with some other studies. Ranaldi et al. (1996) therefore mentioned that the narrow dose range of the inverted-U function may be related to the discrepancies in MK-801 effects on reward that are reported in the literature.

The dose of cocaine being self-administered may also contribute to effects of NMDA receptor antagonists. For example, it has been shown that the self-administration of cocaine (1.0 mg kg^{-1} per IV injection) was not affected by pretreatment with MK-801 (0.01-0.1 mg kg^{-1}) (Pierce et al. 1997). However, using lower doses of cocaine (0.16 or 0.4 mg kg^{-1} per IV injection), MK-801 (0.1 mg kg^{-1}) was shown to reduce responding for cocaine, without altering lever-pressing for food. Pierce et al. (1997) loosely concluded that NMDA receptor blockade "causes rats to work harder for cocaine reinforcement."

The ability of NMDA receptor blockade to interrupt the *acquisition* or development of cocaine self-administration was investigated by Schenk et al. (1993b). In that study, rats pretreated with MK-801 (0.1 or 0.25 mg kg^{-1}) during self-administration training failed to develop a preference for the active lever to attain cocaine (0.25 mg kg^{-1} per IV injection). More recently, Suto et al. (2003) examined the role of intra-VTA Glu antagonism in the acquisition of responding.

In that study, prior exposure to intra-VTA CPP (1.26 ng or 12.61 ng) several days before acquisition of self-administration did not alter subsequent self-administration of cocaine (0.3 mg kg^{-1} per IV injection). Taken together, these studies suggest that NMDA receptor blockade may disrupt acquisition of self-administration, but the VTA is not a relevant site for this effect. It is also noteworthy that prior exposure to intra-VTA amphetamine facilitated subsequent cocaine self-administration, and this effect was blocked if amphetamine was coadministered with CPP (12.61 ng). Suto et al. (2003) concluded that enhancement of cocaine self-administration by amphetamine requires the activation of NMDA receptors in the VTA during amphetamine administration.

Only one study has examined the potential role of the glycine site of the NMDA receptor in drug self-administration. Shoaib et al. (1995) investigated effects the antagonist (+)-HA-966 on self-administration of cocaine (0.33 mg kg^{-1} per IV injection). In that study, (+)-HA-966 (100 or 200 μg ICV) but not lower doses (10 or 30 μg) suppressed responding for cocaine. It is noteworthy that higher doses of (+)-HA-966 also decreased responding for food, suggesting effects of the antagonist may be nonspecific.

One study has explored the effects of dextromethorphan (a drug shown to induce noncompetitive antagonism of NMDA receptors) on cocaine self-administration. Pulvirenti et al. (1997) reported that dextromethorphan (10-50 mg kg^{-1}) dose-dependently reduced responding for cocaine (0.25 mg per IV injection). In addition, under a progressive ratio schedule for cocaine reinforcement, dextromethorphan (25 mg kg^{-1}) reduced the breaking point. Pulvirenti et al. (1997) concluded that dextromethorphan reduces cocaine self-administration and diminishes the absolute rewarding value of the drug. It is noteworthy that potential nonspecific locomotor effects of dextromethorphan were not reported.

An interesting and contemporary paradigm that stems from the drug self-administration literature is the study of drug-seeking behaviour. Briefly, the reinstatement of drug-seeking (lever pressing) following a period of extinction from drug self-administration is considered to be an animal model of drug-induced craving and relapse. Generally, a "priming" injection of the reinforcing drug will reinstate responding. Glu antagonists may also act as priming injections for the reinstatement responding for cocaine. For example, De Vries et al. (1998) examined the ability of MK-801 to reinstate drug-seeking. In that study, rats self-administered cocaine (0.5 mg kg^{-1} per IV injection); following extinction of responding, a priming injection of MK-801 (0.2 mg kg^{-1} but not 0.1 mg kg^{-1}) significantly enhanced responding for the previously cocaine-paired lever, but not the inactive lever, suggesting that NMDA receptor blockade may reinstate responding for cocaine. The reinstatement of cocaine-seeking was investigated further by Bespalov et al. (2000). In that study, rats were trained to self-administer cocaine (0.32 mg kg^{-1} per IV injection) and following extinction were given a priming injection of saline or cocaine. In saline-primed animals, D-CPPene (0.3-3.0 mg kg^{-1}) did not affect responding for either the active or inactive levers, while memantine increased responding for the active and inactive levers (3 or 10 mg kg^{-1} respectively). As such, neither compound reliably reinstated cocaine-seeking behaviour, in contrast to the findings of De Vries et al. (1998).

Cocaine self-administration may also be reinstated by a priming injection of cocaine; this method was employed by Bespalov et al. (2000), Cornish and Kalivas (2000), and See et al. (2001). Bespalov et al. (2000) showed that neither D-CPPene (0.3-3.0 mg kg^{-1}) nor memantine (1-10 mg kg^{-1}) altered the reinstatement of cocaine self-administration (1.0 mg kg^{-1} per IV injection) by a priming injection of cocaine in the absence of cocaine-associated stimuli. Similarly, Cornish and Kalivas (2000) reported that cocaine self-administration (0.5 mg kg^{-1} per IV injection) was extinguished and then reinstated by cocaine (10 mg kg^{-1}). Intra-NAS CPP (0.025 μg) did not affect this cocaine-induced reinstatement, and did not alter responding for the inactive lever. See et al. (2001) also investigated the ability of Glu receptor antagonists to alter reinstatement of drug-seeking by cocaine. Using rats trained to self-administer cocaine (0.33 mg per IV injection), See et al. (2001) demonstrated that neither microinjection of AP5 (1.97 μg) nor a combination of CNQX + AP5 into the basolateral amygdala affected the reinstatement by cocaine. Taken together, these studies suggest that NAS or amygdalar Glu transmission may not be critical for the reinstatement of responding for primary reward.

A recent study by Park et al. (2002) has expanded upon the procedures used to reinstate drug-seeking behaviour. In that study, following extinction of responding for cocaine (0.25 mg per IV injection), drug-seeking was reinstated by intra-NAS or intra-mPFC cocaine (100 μg). The reinstatement by intra-mPFC cocaine was inconsistently affected by intra-NAS AP5 (3.0 μg), likely because intra-NAS AP5 alone (3-30 μg) also reinstated cocaine-seeking behaviour (incidentally, a finding that is in agreement with the results of De Vries et al. 1998). Together, these studies indicate that cocaine's actions in the NAS and the mPFC, in addition to NMDA receptor activity in the NAS, may mediate drug-seeking.

The self-administration studies described above suggest that NMDA receptors in the NAS may modulate cocaine self-administration. For example, as noted by Shoaib et al. (1995), NMDA receptor blockade may increase the reinforcing properties of cocaine or, alternatively, have suppressant effects on locomotor functioning. It is also possible that the increases in cocaine self-administration may reflect the decreased effectiveness of cocaine following intra-NAS NMDA receptor blockade (Pulvirenti et al. 1992).

Several studies have examined the effects of AMPA/kainate receptor antagonism on cocaine self-administration. As described above, the dose of cocaine being self-administered may affect responses to Glu antagonists. Likewise, Pierce et al. (1997) reported that DNQX (3-30 mg kg^{-1}) did not affect self-administration of cocaine (1.0 mg kg^{-1} per IV injection), while self-administration of a lower dose (0.4 mg kg^{-1} per IV injection) was decreased by DNQX (30 mg kg^{-1}). It is noteworthy that this dose of DNQX also reduced lever-pressing for food. This demonstrated importance of drug dose may help to interpret the findings of Cornish et al. (1999). In that study, rats were trained to self-administer cocaine (1.0 mg kg^{-1} per IV injection), and following training, received intra-NAS CNQX prior to the self-administration session. Intra-NAS CNQX (0.023 μg or 0.23 μg) did not affect responding for the active lever, suggesting AMPA/kainate activity in the NAS may not affect cocaine self-administration; however, this finding would be clarified by the use of lower doses of cocaine.

With respect to the *acquisition* of responding for drug self-administration, Suto et al. (2003) examined the facilitation of cocaine self-administration by Glu antagonists. In that study, prior exposure to intra-VTA CNQX (0.035 ng or 0.116 ng) several days before acquisition of self-administration did not alter the subsequent self-administration of cocaine (0.3 mg kg^{-1} per IV injection). It is noteworthy that prior exposure to intra-VTA amphetamine facilitated subsequent cocaine self-administration, and this effect was blocked if amphetamine was coadministered with CNQX (0.116 ng). This study suggests that AMPA/kainate receptor blockade in the NAS does not disrupt the development of cocaine self-administration, which is in contrast to the results obtained with NMDA receptor antagonism (Schenk et al. 1993b). Suto et al. (2003) concluded that the enhancement of cocaine self-administration by amphetamine requires the activation of AMPA/kainate receptors in the VTA during amphetamine administration.

Three studies have explored the role of AMPA/kainate receptor antagonism in the reinstatement of drug-seeking behaviour by a priming injection of cocaine. Cornish and Kalivas (2000) showed that cocaine self-administration (0.5 mg kg^{-1} per IV injection) was extinguished and then reinstated by cocaine (10 mg kg^{-1}) and that intra-NAS administration of CNQX (0.23 µg) completely inhibited this reinstatement without altering responding for the inactive lever. While the NAS may be an important site for this effect, the amygdala may not be. See et al. (2001) showed using rats trained to self-administer cocaine (0.33 mg per IV injection) that neither microinjection of CNQX (0.83 µg) nor combination of CNQX + AP5 into the basolateral amygdala affected reinstatement by cocaine, suggesting that amygdalar Glu transmission may not be critical for the expression of responding for primary reward. As described above, drug-seeking behaviour may also be reinstated by intra-NAS or intra-mPFC cocaine (100 µg) (Park et al. 2002). This reinstatement has also been blocked by intra-NAS CNQX (0.03-0.3 µg). Taken together, these studies of AMPA/kainate receptor blockade suggest that reinstatement of cocaine-seeking is dependent upon AMPA-mediated Glu transmission in the NAS.

A tabulation of the effects of Glu receptor antagonists on cocaine self-administration is shown below. Table 2.4 summarizes the studies that examined effects of acute administration of Glu antagonists on the self-administration of cocaine. Table 2.5 summarizes the studies that have investigated the ability of Glu antagonists to reinstate drug self-administration (i.e., drug-seeking). Lastly, Table

2.6 summarizes the studies that have investigated effects of Glu antagonists on the reinstatement of cocaine self-administration by a priming injection of cocaine.

The main findings from this section of the review can be summarized as follows: 1) when given to animals that are actively self-administering cocaine, NMDA receptor antagonists may increase the rewarding efficacy of cocaine; 2) when given prior to self-administration training, NMDA receptor blockade may prevent the acquisition of cocaine self-administration, and prevent facilitation of this behaviour by pretreatment with amphetamine; 3) AMPA/kainate receptor antagonists may have similar effects, although this is unclear; 4) NMDA receptor blockade may reinstate cocaine-seeking behaviour following extinction; 5) NMDA receptor blockade does not affect reinstatement of cocaine-seeking by a priming injection of cocaine; and 6) blockade of AMPA/kainate receptors in the NAS, but not the amygdala, may prevent the reinstatement of cocaine-seeking by a priming injection of cocaine.

Table 2.4. Review of studies that have investigated the effects of Glu antagonists on cocaine self-administration. NE = no effect.

Cocaine (mg kg^{-1})	Glu Antagonist	Combined Effect	Reference
1.0	MK-801	NE	Pierce et al. 1997
0.06-0.25	MK-801	↓ responding	Schenk et al. (1993a)
0.16-0.4	MK-801	↓ responding	Pierce et al. 1997
0.33	MK-801	↓ responding	Shoaib et al. (1995)
0.5-2.0	MK-801	NE	Schenk et al. (1993a)
0.33	MK-801	NE	Shoaib et al. (1995)
0.25	MK-801	Blocked acquisition	Schenk et al. (1993b)
0.19-1.50	MK-801 (0.05-0.15 mg kg^{-1})	↑ breaking point	Ranaldi et al. (1996)
0.19-1.50	MK-801 (0.2 mg kg^{-1})	↓ breaking point	Ranaldi et al. (1996)
1.0	Intra-NAS MK-801	NE	Cornish et al. 1999
1.0	Intra-NAS AP5	NE	Cornish et al. 1999
0.75	APV	↑ responding	Pulvirenti et al. 1992
0.33	(+)-HA-966	↓ responding	Shoaib et al. (1995)
0.3	Intra-VTA CPP	NE	Suto et al. 2003
0.3	Intra-VTA CPP	Blockade of ↑ responding by amphetamine	Suto et al. 2003
0.25	Dextromethorphan	↓ responding ↓ breaking point	Pulvirenti et al. 1997
1.0	DNQX	NE	Pierce et al. 1997
0.4	DNQX	↓ responding	Pierce et al. 1997
1.0	Intra-NAS CNQX	NE	Cornish et al. 1999
0.3	Intra-VTA CNQX	NE	Suto et al. 2003
0.3	Intra-VTA CNQX	Blockade of ↑ responding by amphetamine	Suto et al. 2003

Table 2.5. Review of studies that have investigated the reinstatement of cocaine self-administration by acute administration of Glu antagonists.

Cocaine (mg kg^{-1})	**Glu Antagonist**	**Reinstatement of responding**	**Reference**
0.50	MK-801	Yes	De Vries et al. 1998
0.32	Memantine	No	Bespalov et al. 2000
0.32	D-CPPene	No	Bespalov et al. 2000
0.25	Intra-NAS AP5	Yes	Park et al. 2002

Table 2.6. Review of studies that have investigated the effects of Glu antagonists on the reinstatement of cocaine self-administration by a priming injection of cocaine. NE = no effect.

Cocaine (mg kg^{-1})	**Glu Antagonist**	**Effect on reinstatement by cocaine**	**Reference**
0.32	Memantine	NE	Bespalov et al. 2000
0.50	Intra-NAS CPP	NE	Cornish and Kalivas 2000
0.32	D-CPPene	NE	Bespalov et al. 2000
0.33	Intra-AMYG AP5	NE	See et al. 2001
0.25	Intra-NAS CNQX	Blockade	Park et al. 2002
0.50	Intra-NAS CNQX	Blockade	Cornish and Kalivas 2000
0.33	Intra-AMYG CNQX	NE	See et al. 2001
0.33	Intra-AMYG CNQX+AP5	NE	See et al. 2001

Place conditioning

There are numerous studies of the interaction between Glu antagonists and DA agonists in the context of place conditioning. Specifically, Glu antagonists have either been coadministered with the drug to be conditioned (to alter the *acquisition* of place conditioning) or administered during post-conditioned testing (to alter the *expression* of place conditioning).

Most studies in this context have used NMDA receptor antagonists. Hoffman (1994) examined the effect of MK-801 on CPP induced by amphetamine (2 mg kg^{-1}). In that study, pretreatment with MK-801 (0.03-0.3 mg kg^{-1}) prior to conditioning did not alter the acquisition of amphetamine-induced CPP. It is noteworthy that MK-801 (0.1 mg kg^{-1}) alone induced CPP, and thus may have masked the ability of NMDA receptor blockade to block the rewarding effect of amphetamine. In contrast to this finding, Cervo and Samanin (1995) showed that when MK-801 was coadministered during conditioning, MK-801 (0.25 mg kg^{-1}, but not 0.1 mg kg^{-1}) blocked the acquisition of CPP induced by cocaine (10 mg kg^{-1}). In addition, when given prior to post-conditioned testing, MK-801 (0.1, 0.25, 0.5 mg kg^{-1}) did not affect the expression of cocaine-induced CPP. Although effects of MK-801 alone were not reported, the authors suggested that NMDA receptor function may be important for the stimulus-reward association in cocaine CPP. Likewise, Kim et al. (1996) showed that cocaine (15 mg kg^{-1}) induced CPP while MK-801 (0.1 mg kg^{-1}) was ineffective, and that MK-801 (0.05 or 0.1 mg kg^{-1}) inhibited the acquisition of CPP induced by cocaine. In agreement with these results, Kim and Jang (1997) reported that MK-801 (0.1 mg kg^{-1}) alone did not show any place conditioning, while methamphetamine (2 mg kg^{-1}) induced CPP. Co-administration of MK-801 with methamphetamine during conditioning reduced, but did not block, the acquisition of methamphetamine-induced CPP. Similar effects have been observed with memantine. Kotlińska and Biała (2000) reported that memantine alone (7.5 mg kg^{-1}) was ineffective while cocaine (5 mg kg^{-1}) induced CPP. Co-administration of memantine (7.5 mg kg^{-1}) during conditioning to cocaine attenuated the acquisition of cocaine-induced CPP. Similarly, acute memantine (7.5 mg kg^{-1}) administered prior to post-conditioned testing prevented the expression of cocaine-induced CPP, suggesting that NMDA receptors may participate in the development and expression of the rewarding properties of cocaine. This concept is reinforced by data showing that competitive NMDA antagonists may also affect expression of CPP. Bespalov (1996) reported that (±)-CPP (20-30 mg kg^{-1}) blocked the expression of CPP induced by amphetamine (1.5 mg kg^{-1}), though effects of (±)-CPP alone were not reported.

The glycine site of the NMDA receptor may be related to the development and expression of conditioned reward. Mead and Stephens (1999) showed that the antagonist L-701,324 (0.3 or 10 mg kg^{-1}) alone was ineffective, but blocked the expression of CPP induced by amphetamine (0.25 mg kg^{-1}). This result may suggest that expression of conditioned responses may be dependent on NMDA receptor-mediated transmission involving the glycine binding site. However, this theory may not be supported by data reported by Kotlińska and Biała (1999) showing that the expression of CPP induced by cocaine (5 mg kg^{-1}) was not affected by acute administration of L-701,324 (2.5 or 5 mg kg^{-1}) before post-conditioned testing. However, in that study the acquisition of CPP induced by cocaine was blocked by co-administration of L-701,324 (2.5 or 5 mg kg^{-1}) during conditioning (Kotlińska and Biała 1999). These studies may indicate that glycine site receptor antagonists may prevent the reinforcing effects of amphetamine, but not cocaine, if such effects have already been developed.

AMPA/kainate receptor antagonists may also affect CPP induced by DA agonists. Layer et al. (1993b) were the first to report that intra-NAS DNQX (1.0 μg) prior to amphetamine (1 mg kg^{-1}) during conditioning completely prevented the acquisition of amphetamine CPP. In a second experiment, intra-NAS DNQX (1.0 μg) before post-conditioned testing completely prevented the expression of amphetamine CPP. It is noteworthy that DNQX alone did not induce place conditioning. Similar results were reported by Kaddis et al. (1995) using cocaine-induced CPP. In that study, intra-NAS DNQX (1.0 μg) blocked the acquisition of CPP to cocaine (20 mg kg^{-1}). When given only prior to post-conditioned testing, and not during the conditioning phase, intra-NAS DNQX (1.0 μg) attenuated the expression of cocaine-induced CPP; DNQX alone was not effective. Cervo and Samanin (1995) also investigated the effects of DNQX on CPP induced by cocaine (10 mg kg^{-1}). When coadministered during conditioning, intracerebroventricular administration of DNQX (1 or 3 μg) did not affect acquisition of cocaine-induced CPP, but DNQX (1 μg) did block the expression of CPP to cocaine during post-conditioned testing. It is noteworthy that the effects of DNQX alone were not reported. While the effects of DNQX on expression of CPP are similar amongst these studies, differences in the effects of DNQX on acquisition of CPP may be related to the route of administration.

Two studies have reported effects of the more selective AMPA antagonist NBQX on place conditioning. Mead and Stephens (1999) reported that administration of NBQX (30 mg kg^{-1}) during conditioning attenuated the acquisition of CPP by amphetamine (0.25 mg kg^{-1}); however NBQX, at that dose, induced CPA when given alone. A lower dose of NBQX was used to assess the expression of amphetamine-induced CPP; NBQX (10 mg kg^{-1}) failed to affect the expression of amphetamine CPP. In contrast, the less-selective AMPA/kainate antagonist CNQX (1, 3, or 10 mg kg^{-1}) blocked the expression of CPP induced by amphetamine. Recent work from this laboratory has explored the effect of NBQX microinjections on place conditioning induced by the $D_{2/3}$ DA agonist 7-OH-DPAT. Biondo (2002) showed that relatively "high" doses of 7-OH-DPAT (5.0 mg kg^{-1}) may induce CPP, and that acquisition of 7-OH-DPAT induced CPP was prevented by intra-NAS shell administration of NBQX (0.5 μg), which alone was ineffective.

Noncompetitive AMPA/kainate receptor antagonists may also affect place conditioning to DA agonists. Tzschentke and Schmidt (1997) showed that amphetamine (2 mg kg^{-1}) induced CPP while GYKI 52466 (3 mg kg^{-1}) was ineffective. The expression of amphetamine CPP was blocked by acute administration of GYKI 52466 (3 mg kg^{-1}) prior to post-conditioned testing, suggesting that noncompetitive AMPA/kainate receptor antagonists may be useful in the treatment of amphetamine abuse.

Taken together, results using AMPA/kainate receptor antagonists in the context of place conditioning indicate that the acquisition and expression of the rewarding properties of DA receptor stimulation may require activation of AMPA/kainate receptors in the NAS.

It is noteworthy that one study has investigated the effects of co-administration of NMDA and non-NMDA antagonists on cocaine-induced CPP. Harris and Aston-Jones (2003) demonstrated that cocaine (10 mg kg^{-1}) induced CPP; acquisition of this effect was blocked by co-administration of intra-VTA AP5 (0.047 μg) + CNQX (0.028 μg) to antagonize both NMDA and non-NMDA receptors. Intra-VTA AP5 + CNQX also induced CPP, indicating the attenuation of cocaine CPP was not due to the antagonists' aversive properties. Harris and Aston-Jones (2003) concluded that activation of both subtypes of Glu receptors in the VTA may be needed to establish the conditioned reinforcing effects of cocaine.

A tabulation of the effects of Glu receptor antagonists and DA agonists on place conditioning is shown in Table 2.7. The main findings from this section of the review can be summarized as follows: 1) amphetamine induces CPP; the acquisition of this effect may be blocked by concomitant AMPA/kainate receptor blockade during conditioning, while expression of this effect may be blocked by

Glu receptor antagonism; 2) cocaine induces CPP; the effects of Glu receptor blockade on this effect are unclear, but acquisition or expression may be prevented; and 3) the acquisition of CPP to methamphetamine or 7-OH-DPAT may be disrupted by NMDA or AMPA/kainate receptor blockade.

Table 2.7. Review of place conditioning studies that have investigated effects of DA agonists and Glu antagonists. NE = no effect, NR = not reported.

DA Agonist (to induce CPP)	Glu Antagonist (Effect)	Combined Effect	Reference
Amphetamine	MK-801 (CPP)	NE on acquisition	Hoffman 1994
	(±)-CPP (NR)	Blocked expression	Bespalov 1996
	Intra-NAS DNQX (NE)	Blocked acquisition Blocked expression	Layer et al. 1993b
	CNQX (NE)	Blocked expression	Mead and Stephens 1999
	NBQX (CPA) 30 mg kg^{-1}	Attenuated acquisition	Mead and Stephens 1999
	NBQX (NE) 10 mg kg^{-1}	NE on expression	Mead and Stephens 1999
	GYKI 52466 (NE)	Blocked expression	Tzschentke and Schmidt 1997
	L-701,324 (NE)	Blocked expression	Mead and Stephens 1999
Cocaine	MK-801 (NR)	Blocked acquisition NE on expression	Cervo and Samanin 1995
	MK-801 (NE)	Attenuated acquisition	Kim et al. 1996
	Memantine (NE)	Attenuated acquisition Blocked expression	Kotlińska and Biała 2000
	ICV DNQX (NR)	NE on acquisition Blocked expression	Cervo and Samanin 1995
	Intra-NAS DNQX (NE)	Blocked acquisition Attenuated expression	Kaddis et al. 1995
	Intra-VTA AP5 + CNQX (CPP)	Blocked acquisition	Harris and Aston-Jones 2003
	L-701,324 (NE)	Blocked acquisition NE on expression	Kotlińska and Biała 1999
Methamphetamine	MK-801 (NE)	Attenuated acquisition	Kim and Jang 1997
7-OH-DPAT	Intra-NAS shell NBQX (NE)	Blocked acquisition	Biondo 2002

Conclusion

Results from studies of locomotor activity, BSR, drug-self administration, and place conditioning indicate that there are widespread interactions between DA and Glu in the context of motivated behaviour. Psychostimulants such as amphetamine and cocaine clearly have the ability to induce locomotor hyperactivity and conditioned place preferences in laboratory animals. A primary goal of the literature that has employed Glu receptor antagonists in combination with psychostimulants is to assess the ability of such compounds to attenuate, or "turn down", the acute stimulant effects or conditioned rewarding effects of psychostimulants, as such compounds may prove useful in the treatment of drug abuse.

Several important conclusions that lead into the objectives of this thesis can be drawn from this review. The studies of primary reinforcement described above suggest that the reward-increasing effect of cocaine in BSR may be potentiated by NMDA receptor blockade, and that NMDA receptor antagonists may increase the rewarding efficacy of self-administered cocaine. Also, NMDA receptor blockade may reinstate cocaine-seeking behaviour following extinction. In addition, the effects of intra-NAS 7-OH-DPAT are potentiated by AMPA/kainate receptor blockade. In the NAS shell, 7-OH-DPAT and AMPA/kainate receptor blockade may act synergistically to decrease reward in BSR. These studies suggest that Glu receptor antagonists and DA agonists may be acting in the same direction (i.e., synergy or potentiation was observed).

In contrast, several of the studies described above suggest that NMDA or AMPA/kainate receptor blockade may be acting in a direction opposite to DA receptor stimulation in the context of motivation. For example, in locomotor activity studies, amphetamine or cocaine-induced hyperactivity is attenuated by Glu receptor blockade and may be blocked by intra-NAS AMPA/kainate antagonists. Also, the effects of intra-NAS D_1 DA receptor stimulation may be blocked by intra-NAS Glu receptor blockade. The antagonism of AMPA/kainate receptors in the NAS, but not the amygdala, may prevent the reinstatement of cocaine-seeking by a priming injection of cocaine. Lastly, the acquisition of the conditioned reinforcing effects of amphetamine or cocaine may be blocked by concomitant AMPA/kainate receptor blockade. These studies suggest that Glu antagonists and DA agonists may be acting in opposite directions; or in other words, that stimulation of Glu receptors may be required for the motivational changes induced by DA agonists.

Regardless of these differing directions of DA-Glu interactions, the contents of this chapter indicate that the vast majority of prior studies of reward-related behaviour have used nonspecific or nonselective DA agonists to alter the animals' observed state of motivation or reward. While the use (and abuse) of broad DA agonists is of key importance to the rationale of this thesis, our understanding of reward pathways in the CNS would be greatly clarified by the use of more selective DA agonists. Therefore, the experiments that follow will explore interactions between Glu receptor antagonism and relatively selective stimulation of D_2-like DA receptors. As such, the minority of studies described above that have used 7-OH-DPAT or quinpirole are of particular usefulness for the interpretation of the following series of experiments.

Thesis objectives

As described in Chapter 1, Glu NMDA and AMPA receptors have been implicated in the pathophysiology of schizophrenia (Bunney et al. 1995). In addition, NMDA receptor antagonists and AMPA receptor antagonists may differentially affect behaviour (Hauber and Andersen 1993). Previous work from this laboratory has shown synergistic effects of administration of an AMPA/kainate receptor antagonist and $D_{2/3}$ DA receptor stimulation in the NAS on locomotor activity (Choi et al. 2000). In addition, Biondo (2002) showed that systemic $D_{2/3}$ DA receptor stimulation may induce conditioned place preference that is abolished by AMPA receptor blockade in the NAS. Therefore, this thesis aimed to explore further the effects of Glu receptor antagonism on behavioural responses induced by selective DA receptor agonists.

Based on these observations, the objectives of this thesis were as follows:

(1) To assess the conditioned reinforcing effects of NMDA receptor blockade in the context of place conditioning.

(2) To replicate conditioned reinforcing effects of $D_{2/3}$ DA receptor stimulation (Biondo 2002) and determine if these effects are altered by simultaneous blockade of NMDA receptors.

(3) To assess whether locomotor stimulation induced by NMDA receptor blockade is differentially affected by postsynaptic D_3 vs. presynaptic D_2 DA receptor stimulation, and determine the contribution of the NAS in this context.

(4) To explore the interaction between systemic NMDA receptor blockade and postsynaptic D_3 vs. presynaptic D_2 DA receptor stimulation, on electrical self-stimulation of the VTA.

(5) To replicate the synergistic reward-decreasing effects of AMPA/kainate receptor blockade and $D_{2/3}$ DA receptor stimulation in the NAS on electrical self-stimulation of the VTA, and explore whether similar effects are observed when an NMDA antagonist is used in place of an AMPA/kainate receptor antagonist.

Chapter 3. Locomotor stimulation induced by the NMDA receptor antagonist MK-801 is differentially affected by D_2-like DA receptor stimulation

(A version of this chapter has been accepted for publication in *Neuropharmacology* – see Clements & Greenshaw 2005a)

Introduction

Glu receptor antagonists, such as phencyclidine (PCP), induce psychosis and symptoms resembling schizophrenia in humans (Luby et al. 1959; Snyder 1980; Javitt and Zukin 1991). In addition, PCP and other Glu antagonists such as MK-801 also induce hyperactivity in laboratory animals (Verebey et al. 1981; Ford et al. 1989). Spontaneous exploration of the environment is a complex behaviour that is affected by sensory processing, environmental cues, motor responses, and the animals' state of arousal (Kelley et al. 1989). The purpose of this work was to explore DA-Glu interactions in the mesolimbic system by investigating effects of systemic or intra-NAS administration of DA and Glu subtype-specific ligands on spontaneous locomotor activity.

As described in Chapter 1, DA receptors are currently divided into two families, D_1-like (D_1 and D_5) and D_2-like (D_2, D_3, D_4). The mesolimbic DA system is of primary interest in the investigation of DA-Glu interactions in motivation, as DA-containing cell bodies of the VTA terminate in the NAS, as do Glu projections from the prefrontal cortex (Sesack and Pickel 1992; Pennartz et al. 1994). Within the NAS, Deutch et al. (1993) have suggested that the NAS shell subregion, rather than core, is likely more associated with reward-related processes; however recent evidence may also implicate the NAS core in reward-related learning (Hatip-Al-Khatib et al. 2001; Cardinal et al. 2004; Li et al. 2004; Giertler et al. 2005). Of primary interest in psychiatric disorders of motivation, D_3 DA receptors are preferentially localized within terminal areas of the limbic forebrain (including the NAS shell subregion and VTA) that are known to be associated with cognition, emotional processes, and endocrine functions (Sokoloff et al. 1990; Lévesque et al. 1992; Diaz et al. 2000). In addition, antipsychotic medications such as risperidone have been shown to demonstrate some binding affinity for the D_3 DA receptor (for review, see Schwartz et al. 2000). The D_3 DA receptor subtype may therefore be a useful target in defining the pathophysiology of, and treatments for, schizophrenia and drug abuse.

To explore interactions between Glu and DA in this brain region, we have employed two dissimilar DA agonists, apomorphine and 7-OH-DPAT (see Chapter 1). Whereas the dose of apomorphine used in the present study induces hypoactivity by presumably stimulating primarily D_2 DA autoreceptors (see page 24), the systemic dose of 7-OH-DPAT stimulates primarily D_3 DA receptors that may be located postsynaptically. In addition, it has previously been shown that intra-NAS 7-OH-DPAT decreases spontaneous locomotor activity of habituated rats (e.g., Choi et al. 2000). Taken together, comparative effects of apomorphine and 7-OH-DPAT may contribute to the differentiation of pre- vs. postsynaptic interactions within the mesolimbic system.

As described in Chapter 1, Glu receptors are classified into four classes: NMDA, AMPA, kainate, and G-protein coupled metabotropic receptors. MK-801 is a noncompetitive NMDA receptor antagonist (Wong et al. 1986) that induces dose-dependent hyperactivity (e.g., Ford et al. 1989; Maj et al. 1991) that may be DA-dependent (Dall'Olio et al. 1992; Ouagazzal et al. 1993; Bristow et al. 1993). However, the precise neurochemical interaction between NMDA receptor blockade and DA receptor activity in the NAS remains unclear.

The current investigation of NMDA receptor – DA receptor interactions in the context of spontaneous exploration complements our prior studies using AMPA/kainate receptor antagonists. Recall (as described in Chapter 2) that intra-NAS AMPA/kainate receptor blockade has been shown to potentiate a decrease in activity induced by intra-NAS 7-OH-DPAT (Choi 2000; Choi et al. 2000). The objectives of this study were: 1) to explore the potential interaction between NMDA receptor blockade and $D_{2/3}$ DA receptor stimulation, both systemically and in the NAS shell subregion; and 2) to assess the interaction between NMDA receptor blockade and presumably presynaptic D_2 DA receptor stimulation by low dose apomorphine. Based on the previous results showing that AMPA/kainate receptor blockade potentiated hypoactivity induced by 7-OH-DPAT, it was hypothesized that NMDA receptor blockade may potentiate the effects of D_2-like DA receptor stimulation.

Hypotheses

The hypotheses for experiments described in this chapter were as follows:

1. Systemic or intra-NAS shell MK-801 alone would increase activity.
2. Systemic apomorphine alone would decrease activity.
3. Systemic or intra-NAS shell 7-OH-DPAT would decrease activity.
4. MK-801 would potentiate the locomotor-suppressant effects of apomorphine.
5. MK-801 would potentiate locomotor-suppressant effects of 7-OH-DPAT, both systemically and in the NAS shell.

Materials and methods

Animals

Male Sprague-Dawley rats (200-250 g; Health Sciences Laboratory Animal Services, University of Alberta) were housed individually in a temperature- (21±1°C) and humidity-controlled environment with a 12-hour light/dark cycle (lights on 0700-1900 hours). Food (LabDiet 5001 Rodent Diet, PMI Nutrition International Inc., Brentwood, MO, USA) and water were freely available. Separate groups of animals were used for each experiment, and testing always occurred between 0900-1700 hours. The care and use of animals was approved by the Health Sciences Animal Policy and Welfare Committee and the University Animal Policy and Welfare Committee at the University of Alberta, and complied with the "Guide to the Care and Use of Experimental Animals"

(Volume 1, 2nd Edition, 1993; Volume 2, 1984) published by the Canadian Council on Animal Care (CCAC).

Drugs

(+)-MK-801 maleate [dizocilpine; (5S, 10R)-(+)-5-methyl-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5,10-imine maleate], 7-OH-DPAT ((±)-7-hydroxy-2-dipropylaminotetralin) hydrobromide, and apomorphine hydrochloride hemi-hydrate were obtained from Sigma RBI (Oakville, ON, CA). Compounds were dissolved in isotonic saline (Fisher Scientific Ltd., Nepean, ON, CA) and drug solutions were made daily (pH 5.0-7.0) according to the protocol in Appendix A. Artificial cerebrospinal fluid (CSF) was freshly prepared according to Lewis and Elliott (1950) (see Appendix B). Doses are expressed as free bases. In experiments 1 and 2, MK-801 (0.03, 0.06, or 0.13 mg kg^{-1}) was given IP 30 minutes prior while apomorphine (experiment 1; 0.05 mg kg^{-1}) or 7-OH-DPAT (experiment 2; 0.03 mg kg^{-1}) was given SC 10 or 15 minutes prior respectively. All compounds were given in a volume of 1.0 mL kg^{-1}. In experiment 3, MK-801 (0.66 μg) and 7-OH-DPAT (5.0 μg) were microinjected immediately prior to testing (see below). The dose of 7-OH-DPAT used in these experiments was based upon prior work in this laboratory (Choi et al. 2000; Biondo 2002). Animals received a randomized series of eight systemic drug administrations (experiments 1 and 2) or four central microinjections (experiment 3) according to a repeated measures design with 3 days of baseline testing between treatments.

Stereotaxic surgery

A standard operating procedure for central implantation is provided in Appendix C. Briefly, under aseptic conditions, animals weighing between 300-350 g were anaesthetized using Halothane (2-bromo-2-chloro-1,1,1-trifluoroethane; Halocarbon Laboratories, River Edge, NJ, USA) and placed in a Kopf stereotaxic instrument (Kopf Instruments, Tujunga, CA, USA). Animals were implanted bilaterally with stainless steel 22 gauge guide cannulae (C313G, Plastics One Inc., Roanoke, VA, USA) directed at the NAS shell subregion. Stereotaxic coordinates were interpolated from the target site (defined by Paxinos and Watson 1986) and placed at angles from the sagittal plane to avoid damage to blood sinuses and cerebral ventricles (Greenshaw 1997b). These coordinates are relative to inter-aural zero with the incisor bar set 2.4 mm below the inter-aural line: AP +10.1 mm, LM ±0.2 mm, DV +3.7 mm, 16° lateral to the sagittal plane. Tips of guide cannulae were placed 1.0 mm above the target site. Cannulae were fixed to the skull using stainless steel screws (Lomat Watch Co., Montréal, QC, CA) and dental acrylic (Caulk/Dentsply, Milford, DE, USA). Dummy cannulae (C313DC, Plastics One Inc., Roanoke, VA, USA) were left in place between microinjection procedures to prevent occlusion. Testing began at least one week after surgery.

Apparatus

Spontaneous locomotor activity was measured using computer-monitored photobeam boxes (I. Halvorsen System Design, Phoenix, AZ, USA) that consisted of Plexiglas test cages (43 cm L x 43 cm W x 30 cm H). A 12 x 12 array of horizontal infrared beams permitted recording of both horizontal and consecutive beam breaks. While horizontal activity includes any movement about the array and is an overall measure of activity, consecutive activity includes any repetitive movement (i.e., immediately consecutive beam breaks). Vertical activity (rearing behaviour) was measured using 12 additional beams 12 cm above, and parallel to, the floor. Drugs that induce stereotypy or alter the animals' rearing behaviour will be detected using consecutive or vertical measurements respectively, while an overall depiction of generalized activity is provided by horizontal measurements. Local time course data for each measure (horizontal, consecutive, and vertical) were automatically recorded during 5 minute intervals for the duration of the test period. The test environment was illuminated with light extending into the visible red frequency in accordance with prior studies in this laboratory.

Behavioural testing procedure

Animals were handled and habituated to the photobeam boxes for 5 consecutive days (60 min per day). Throughout the experiment, each animal was always exposed to the same apparatus that was cleaned thoroughly between animals using diluted ammonia-based window cleaner (1:6 in water) (No Name

Glass Cleaner with Ammonia, Loblaw Companies Ltd., Toronto, ON). Experiment 1 (n=6) examined effects of MK-801 (0, 0.03, 0.06, 0.13 mg kg^{-1}), apomorphine (0.05 mg kg^{-1}), and each combination of MK-801 + apomorphine. Experiment 2 (n=8) examined effects of MK-801 (0, 0.03, 0.06, 0.13 mg kg^{-1}), 7-OH-DPAT (0.03 mg kg^{-1}), and each combination of MK-801 + 7-OH-DPAT. Experiment 3 (n=7) examined intra-NAS shell effects of CSF, MK-801 (0.66 μg), 7-OH-DPAT (5.0 μg), and combination of MK-801 + 7-OH-DPAT. Routes and timing of drug administration are described above under the heading *Drugs*. All experiments consisted of a repeated measures design to minimize the number of animals required.

Microinjection procedure

For each microinjection, dummy cannulae were carefully removed and 28 gauge injection cannulae (C313I, Plastics One Inc., Roanoke, VA, USA) were slowly lowered into the guide cannulae. The tip of each injection cannula extended 1.0 mm beyond the tip of the guide cannula. Bilateral microinjections (0.5 μL per side at a rate of 0.2 μL per min) were delivered over 2.5 minutes using 0.03 mL per meter Accu-rated pump tubes (Fisher Scientific Ltd., Nepean, ON, CA) and 10 μL glass microsyringes (Hewlett-Packard, Mississauga, ON, CA) attached to a Bee Hive Controller (Bioanalytical Systems Inc., West Lafayette, IN, USA). The injection cannulae remained in place for one minute following infusion to allow for drug absorption and diffusion. Microinjection was immediately followed by behavioural procedures.

Histology

Animals with intra-NAS shell cannulae were deeply anaesthetized with sodium pentobarbital (Bimeda-MTC Animal Health Inc., Cambridge, ON, CA; Somnotol or Euthanyl) and perfused intracardially with 50 mL ice-cold isotonic saline (Fisher Scientific Ltd., Nepean, ON, CA) followed by 50 mL 10% w/v buffered formalin phosphate (Fisher Scientific Ltd., Nepean, ON, CA). Following decapitation, brains were removed and stored at room temperature in 10% w/v buffered formalin phosphate for 4-6 hours, then stored in 30% sucrose/10 mM PBS buffer for at least 24 hours. Brains were then flash-frozen by immersion in isopentane (2-methylbutane; Sigma-Aldrich Co., Oakville, ON, CA) chilled on solid carbon dioxide (Praxair Canada Inc., Edmonton, AB, CA). Brains were briefly dried and stored at –80°C until sectioning. Cannula placements were verified by inspection of 40 µm coronal brain sections. This histology protocol is described further in Appendix D. Only animals with correct cannulae placements were included in data analyses, and are equivalent to those presented previously (Choi et al. 2000). A representative photomicrograph showing intra-NAS shell infusion sites is shown in Appendix E.

Statistical analyses

In experiment 1, effects of MK-801 and apomorphine were assessed using a three-way repeated measures ANOVA (MK-801 x apomorphine x time). The effect of MK-801 was explored further by comparing each dose with vehicle using a Newman-Keuls post-hoc test. In experiment 2, effects of MK-801 and 7-OH-DPAT were assessed using a repeated measures three-way ANOVA (MK-801 x 7-OH-DPAT x time). Following significant MK-801 x 7-OH-DPAT interaction, effects of 7-OH-DPAT were explored using matched-sample t-tests with Bonferroni adjustments, and effects of MK-801 were explored using two separate one-way repeated measures ANOVA (7-OH-DPAT present and absent, respectively) followed by comparison of each dose with vehicle using a Newman-Keuls post-hoc test. In experiment 3, effects of MK-801 and 7-OH-DPAT were assessed using a three-way repeated measures ANOVA (MK-801 x 7-OH-DPAT x time). Following observation of a significant MK-801 x 7-OH-DPAT interaction, effects of each compound were explored using matched-sample t-tests with Bonferroni adjustments.

In all experiments, local time-course data were analysed using a one-way repeated measures ANOVA across all treatments for each 5 minute interval. A significant F ratio ($P<0.05$) on a single interval was followed by comparison of each drug with vehicle using a Newman-Keuls post-hoc test. Statistical analyses ($\alpha=0.05$) were completed using statistical software (SPSS Inc., Chicago, IL, USA).

For each repeated measures experiment, Greenhouse-Geisser adjusted degrees of freedom have been used; because of this, degrees of freedom values may appear as non-integers.

Results

Experiment 1: Systemic MK-801 and apomorphine

The general pattern of results indicates that there was no interaction between locomotor hyperactivity induced by MK-801 and locomotor hypoactivity induced by low dose apomorphine. When combined, effects of MK-801 and low dose apomorphine were additive.

Horizontal activity

Three-way repeated measures ANOVA revealed significant effects of MK-801 (F(1.303, 6.515)=9.856, P=0.015) and apomorphine (F(1,5)=7.619, P=0.040), but no significant MK-801 x apomorphine interaction on horizontal activity (F(1.270, 6.350)=0.839, P=0.422) (Figure 3.1(A)). In addition, there was an effect of time (F(1.789, 8.943)=14.276, P=0.002) and a significant apomorphine x time interaction (F(2.560, 12.799)=10.437, P=0.001) but no significant MK-801 x time (F(3.965, 19.823)=1.649, P=0.202) nor MK-801 x apomorphine x time interaction (F(4.091, 20.454)=0.963, P=0.451).

Further analysis of the effect of MK-801 (Figure 3.2) showed that the highest dose significantly increased activity relative to control treatment (Newman-Keuls P<0.05). Analysis of local time course revealed no significant differences between each treatment and saline-treated control animals at any time point (Figure 3.1(B)).

Figure 3.1. Effects of MK-801 and apomorphine (0.05 mg kg^{-1}) on total horizontal locomotor activity (A) (mean ± SEM; n=6). The local time course data of horizontal activity (B) are shown as means (SEM omitted for clarity).

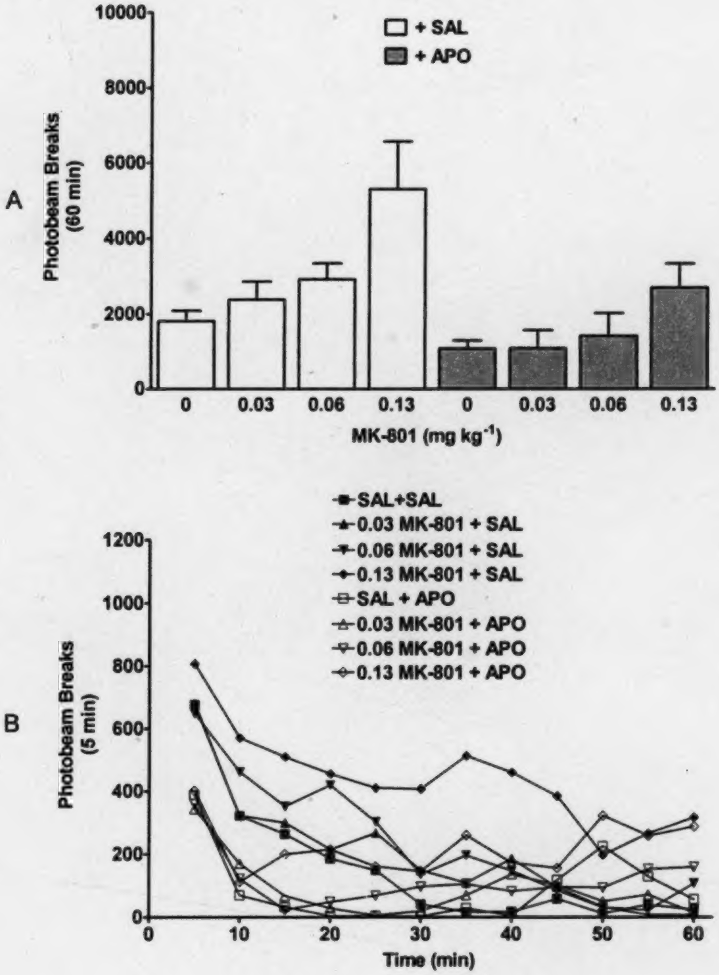
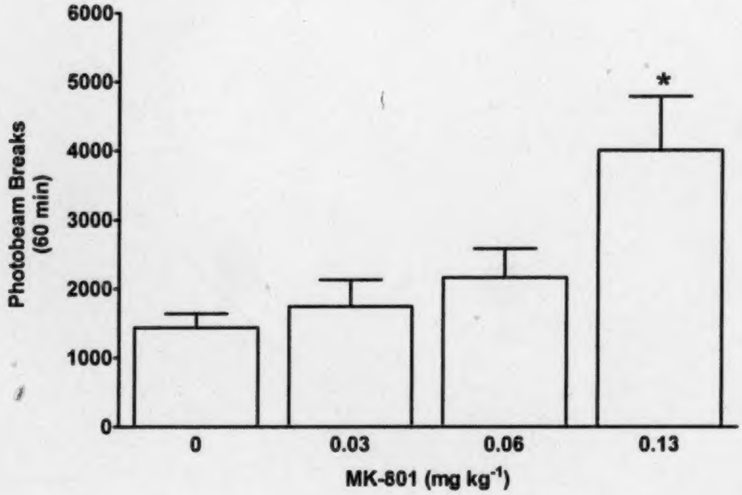


Figure 3.2. Further analysis of the main effect of MK-801 on total horizontal locomotor activity (mean ± SEM; n=12). Significant difference (*) from control at P<0.05.



Consecutive activity

Three-way repeated measures ANOVA revealed a significant effect of apomorphine (F(1,5)=7.916, P=0.037), but not of MK-801 (F(1.448, 7.241)=2.824, P=0.131); nor was there significant MK-801 x apomorphine interaction on consecutive activity (F(1.884, 9.419)=0.196, P=0.813) (Figure 3.3(A)). In addition, there was an effect of time (F(3.722, 18.608)=7.062, P=0.001) and a significant apomorphine x time interaction (F(2.490, 12.451)=4.397, P=0.030) but no significant MK-801 x time (F(4.375, 21.874)=1.189, P=0.345) nor MK-801 x apomorphine x time interaction (F(4.400, 21.999)=1.021, P=0.423). Analysis of local time course revealed no significant differences from saline-treated control animals at any time point (Figure 3.3(B)).

Vertical activity

Three-way repeated measures ANOVA failed to reveal significant effects of MK-801 (F(2.044, 10.219)=1.095, P=0.372) or apomorphine (F(1,5)=6.495, P=0.051) and showed there was no significant MK-801 x apomorphine interaction on vertical activity (F(2.483, 12.414)=0.311, P=0.782) (Figure 3.4(A)). In addition, there was an effect of time (F(1.240, 6.198)=11.921, P=0.011) and a significant apomorphine x time interaction (F(2.457, 12.286)=6.406, P=0.010) but no significant MK-801 x time (F(2.717, 13.583)=2.684, P=0.092) nor MK-801 x apomorphine x time interaction (F(2.459, 12.295)=2.226, P=0.143). Analysis of local time course revealed no significant differences from saline-treated control animals at any time point (Figure 3.4(B)).

Figure 3.3. Effects of MK-801 and apomorphine (0.05 mg kg^{-1}) on total consecutive locomotor activity (A) (mean ± SEM; n=6). The local time course data of consecutive activity (B) are shown as means (SEM omitted for clarity).

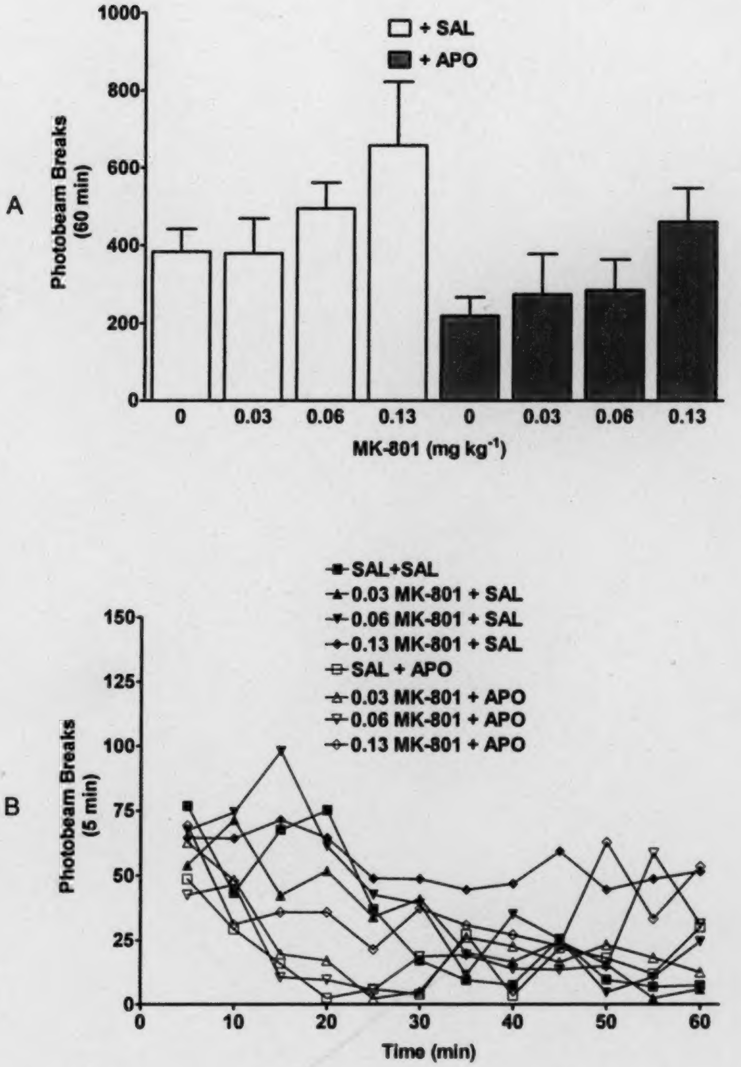
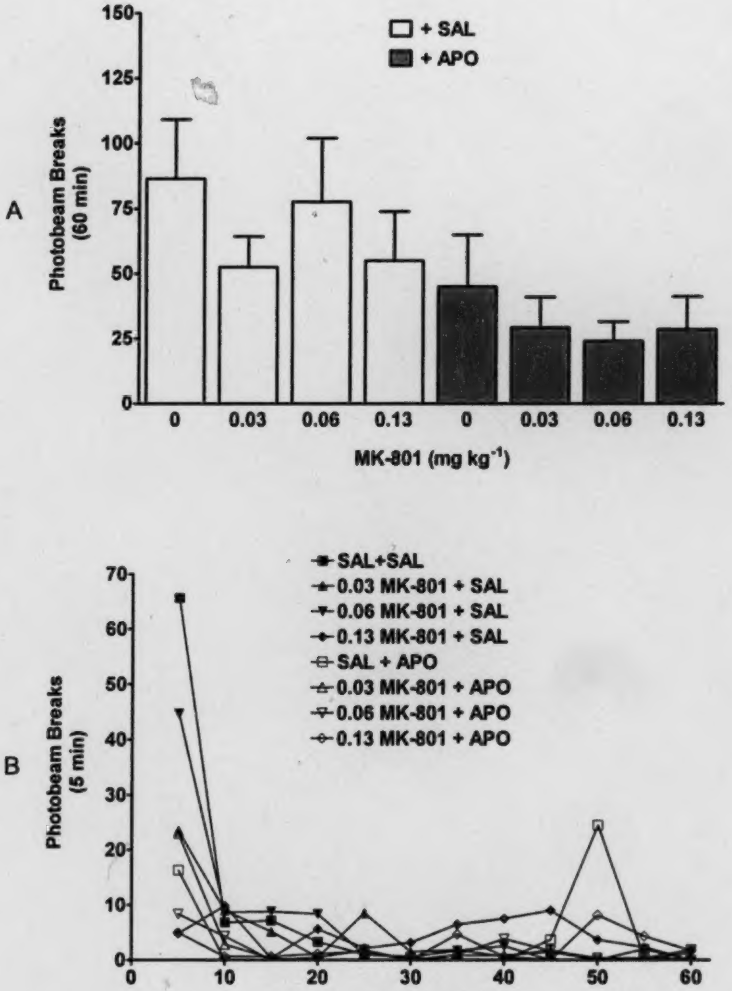


Figure 3.4. Effects of MK-801 and apomorphine (0.05 mg kg^{-1}) on total vertical locomotor activity (A) (mean ± SEM; n=6). The local time course data of vertical activity (B) are shown as means (SEM omitted for clarity).



Experiment 2: Systemic MK-801 and 7-OH-DPAT

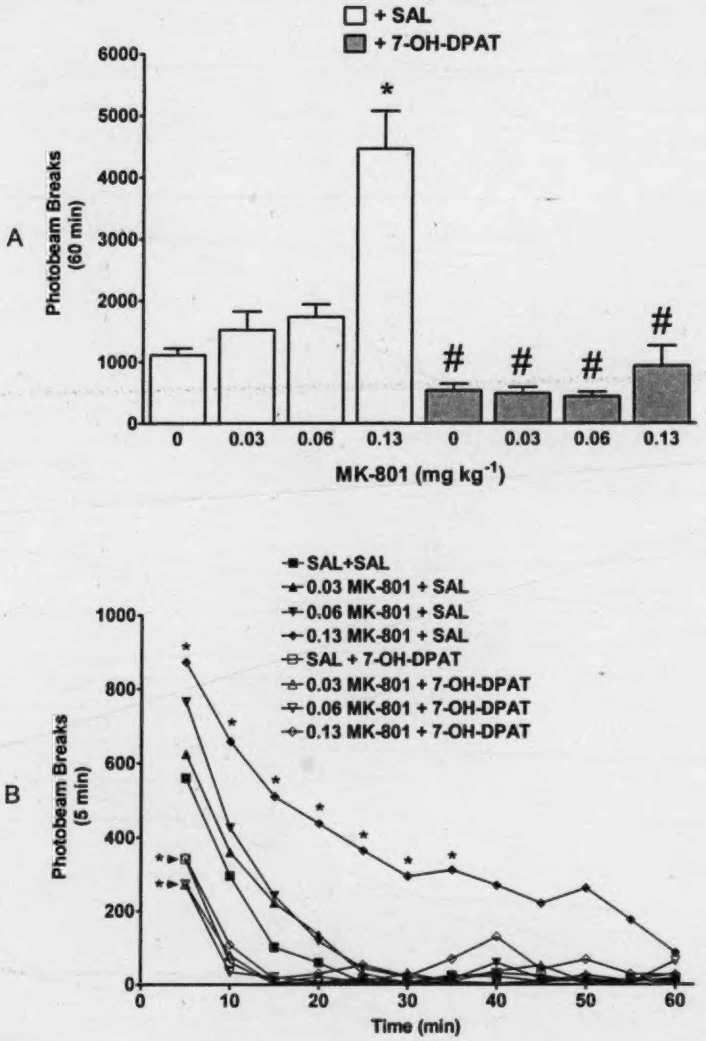
The general pattern of results indicates that there was a significant interaction between effects of MK-801 and 7-OH-DPAT. Locomotor hyperactivity induced by MK-801 was blocked by 7-OH-DPAT.

Horizontal activity

Three-way repeated measures ANOVA revealed significant effects of MK-801 (F(1.391, 9.740)=16.396, P=0.001) and 7-OH-DPAT (F(1,7)=54.763, P<0.001), and a significant MK-801 x 7-OH-DPAT interaction on horizontal activity (F(1.502,10.514)=16.348, P=0.001) (Figure 3.5(A)). In addition, there was an effect of time (F(2.876, 20.130)=103.146, P<0.001) and a significant 7-OH-DPAT x time interaction (F(3.232, 22.625)=37.924, P<0.001) but no significant MK-801 x time (F(5.218, 36.527)=1.229, P=0.315) or MK-801 x 7-OH-DPAT x time interaction (F(4.546, 31.819)=1.911, P=0.126). Further analysis of the effect of MK-801 showed that the highest dose (0.13 mg kg^{-1}) significantly increased activity (Newman-Keuls P<0.05) but was ineffective in the presence of 7-OH-DPAT. In addition, 7-OH-DPAT decreased activity at each dose of MK-801 (MK-801 0 mg kg^{-1}: t(7)=2.821, P=0.026; 0.03 mg kg^{-1}: t(7)=4.606, P=0.002; 0.06 mg kg^{-1}: t(7)=6.030, P=0.001; 0.13 mg kg^{-1}: t(7)=5.820, P=0.001).

Analysis of local time course (Figure 3.5(B)) revealed that MK-801 (0.13 mg kg^{-1}) alone significantly increased activity at 5-35 minutes; in addition, animals with 7-OH-DPAT alone or MK-801 + 7-OH-DPAT showed significantly decreased activity during the first 5 minutes relative to saline-treated control animals (Newman-Keuls $P<0.05$).

Figure 3.5. Effects of MK-801 and 7-OH-DPAT (0.03 mg kg^{-1}) on total horizontal activity (A) (mean ± SEM; n=8). Significant difference (*) from control at $P<0.05$. Significant difference (#) from that dose of MK-801 alone at $P<0.05$. The local time course data of horizontal activity (B) are shown as means (SEM omitted for clarity). Significant difference (*) from control at $P<0.05$.



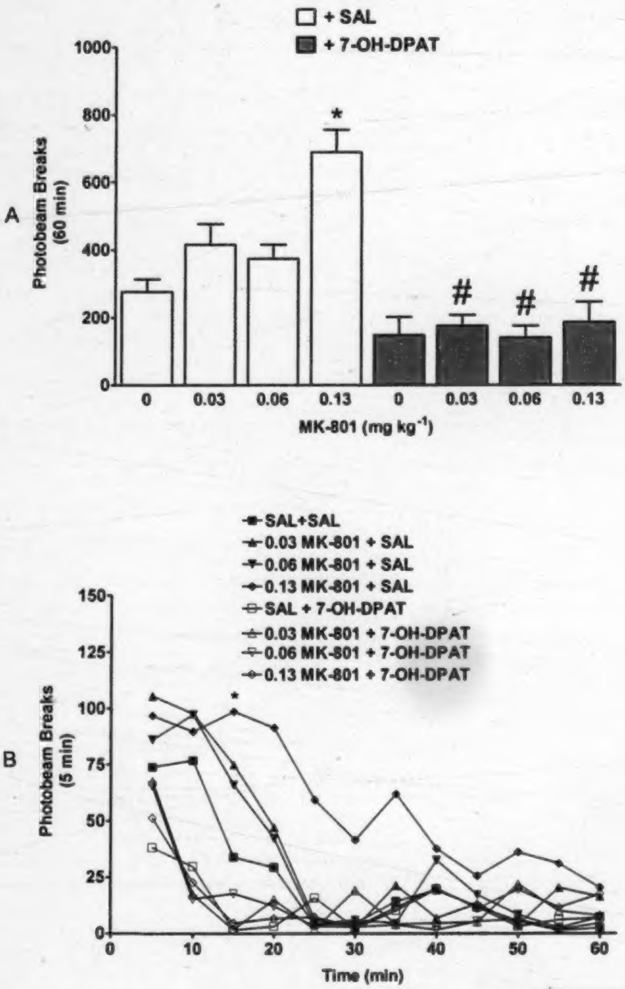
Consecutive activity

Three-way repeated measures ANOVA revealed significant effects of MK-801 (F(1.873, 13.110)=9.007, P=0.004) and 7-OH-DPAT (F(1,7)=27.005, P=0.001), and a significant MK-801 x 7-OH-DPAT interaction on consecutive activity (F(2.056, 14.392)=17.680, P<0.001) (Figure 3.6(A)). In addition, there was an effect of time (F(3.629, 25.404)=41.355, P<0.001) and a significant 7-OH-DPAT x time interaction (F(3.721, 26.046)=13.380, P<0.001) but no significant MK-801 x time (F(5.568, 38.977)=1.296, P=0.283) or MK-801 x 7-OH-DPAT x time interaction (F(5.500, 38.500)=1.113, P=0.371).

Further analysis of the effect of MK-801 showed that the highest dose (0.13 mg kg^{-1}) significantly increased activity (Newman-Keuls P<0.05) but was ineffective in the presence of 7-OH-DPAT. In addition, 7-OH-DPAT decreased activity in the presence of each dose of MK-801 (0.03 mg kg^{-1}: t(7)=4.126, P=0.004; 0.06 mg kg^{-1}: t(7)=4.201, P=0.004; 0.13 mg kg^{-1}: t(7)=7.896, P<0.001).

Analysis of local time course (Figure 3.6(B)) revealed that MK-801 (0.13 mg kg^{-1}) alone significantly increased activity at 15 minutes relative to saline-treated control animals (Newman-Keuls P<0.05).

Figure 3.6. Effects of MK-801 and 7-OH-DPAT (0.03 mg kg^{-1}) on total consecutive activity (A) (mean ± SEM; n=8). Significant difference (*) from control at $P<0.05$. Significant difference (#) from that dose of MK-801 alone at $P<0.05$. The local time course data of consecutive activity (B) are shown as means (SEM omitted for clarity). Significant difference (*) from control at $P<0.05$.

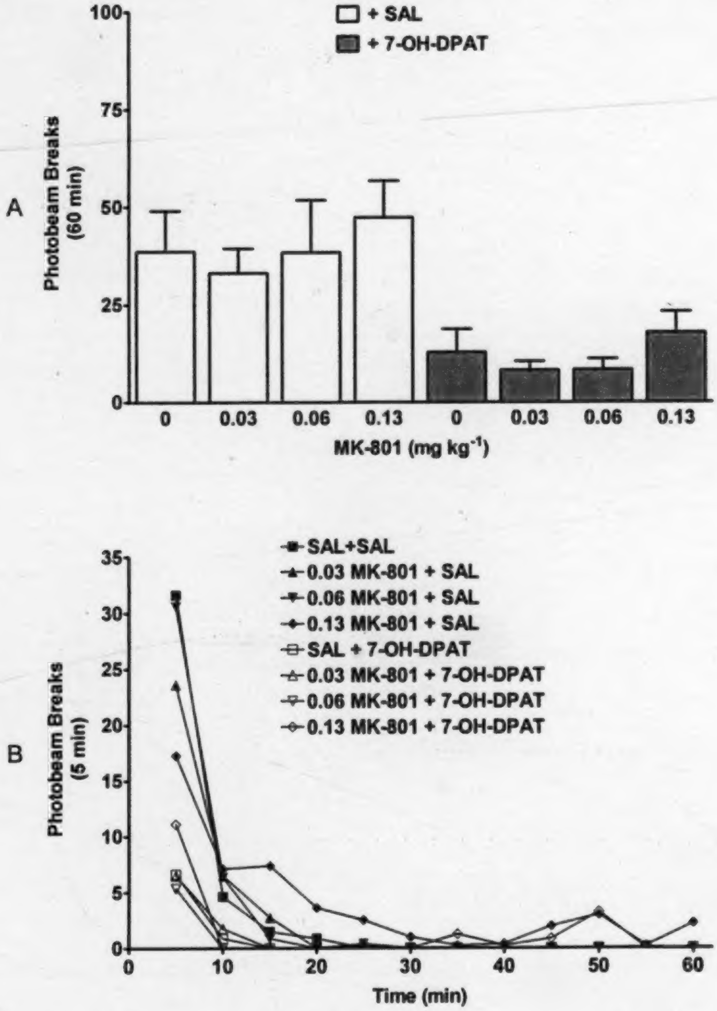


Vertical activity

Three-way repeated measures ANOVA revealed a significant effect of 7-OH-DPAT (F(1,7)=15.802, P=0.005), but not of MK-801 (F(2.228, 15.593)=1.423, P=0.272), nor was there a significant MK-801 x 7-OH-DPAT interaction on vertical activity (F(2.405, 16.833)=0.168, P=0.881) (Figure 3.7(A)). In addition, there was an effect of time (F(1.250, 8.753)=45.153, P<0.001) and a significant 7-OH-DPAT x time interaction (F(1.190, 8.329)=10.461, P=0.009) but no significant MK-801 x time (F(2.193, 15.353)=0.621, P=0.565) or MK-801 x 7-OH-DPAT x time interaction (F(2.016, 14.110)=1.214, P=0.327).

Analysis of local time course revealed no significant differences between each treatment and saline-treated control animals at any time point (Figure 3.7(B)).

Figure 3.7. Effects of MK-801 and 7-OH-DPAT (0.03 mg kg^{-1}) on total vertical activity (A) (mean ± SEM; n=8). The local time course data of vertical activity (B) are shown as means (SEM omitted for clarity).



Experiment 3: Intra-NAS shell MK-801 and 7-OH-DPAT

Histological locations of cannula placements are shown in Figure 3.8.

Consistent with systemic results, the general pattern of results indicates that there was a significant interaction between MK-801 and 7-OH-DPAT. Locomotor hyperactivity induced by MK-801 was blocked by 7-OH-DPAT.

Horizontal activity

Three-way repeated measures ANOVA revealed a significant main effect of MK-801 (F(1,6)=38.313, P=0.001) but not of 7-OH-DPAT (F(1,6)=4.576, P=0.076), and a significant MK-801 x 7-OH-DPAT interaction (F(1,6)=32.858, P=0.001) (Figure 3.9(A)). There was no effect of time (F(1.670, 10.021)=0.962, P=0.399), but significant MK-801 x time and 7-OH-DPAT x time interactions (F(2.465, 14.790)=8.050, P=0.003 and F(2.367, 14.200)=4.019, P=0.036 respectively) without significant MK-801 x 7-OH-DPAT x time interaction (F(2.704, 16.223)=1.862, P=0.179).

Further analysis showed that MK-801 alone significantly increased activity (t(6)=12.096, P<0.001) but was ineffective in the presence of 7-OH-DPAT (t(6)=1.537, P=0.175). 7-OH-DPAT alone was without effect (t(6)=0.638, P=0.547) but decreased activity in the presence of MK-801 (t(6)=4.606, P=0.004).

Analysis of local time course (Figure 3.9(B)) revealed that that MK-801 alone significantly increased activity at 15, 25, and 30 minutes; 7-OH-DPAT alone significantly decreased activity during the first 5 minutes; and co-administration of MK-801 and 7-OH-DPAT initially significantly decreased activity during the first 5 minutes then increased activity at 25 minutes relative to CSF-treated control animals (Newman-Keuls $P<0.05$).

Figure 3.8. Illustration of histological verification of microinjection sites in the NAS shell subregion. Numbers represent distances in the coronal plane from interaural zero according to the atlas of Paxinos and Watson (1986).

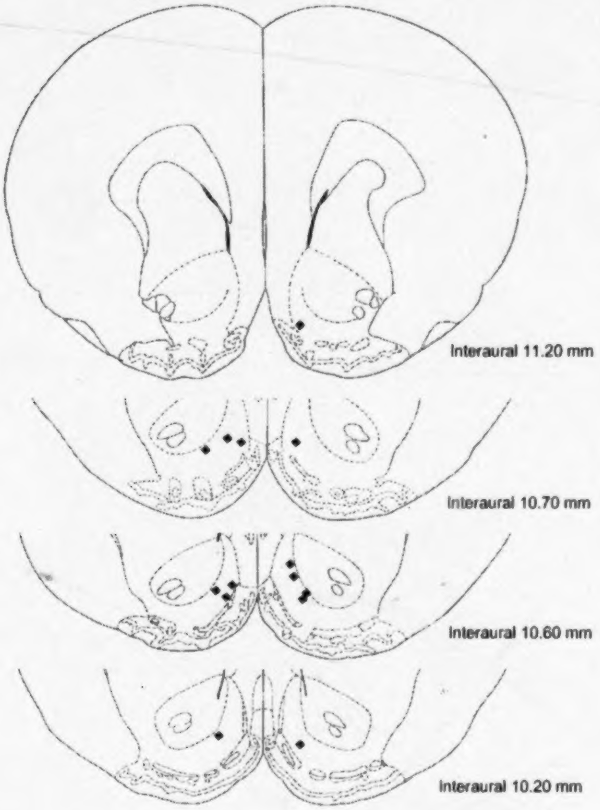
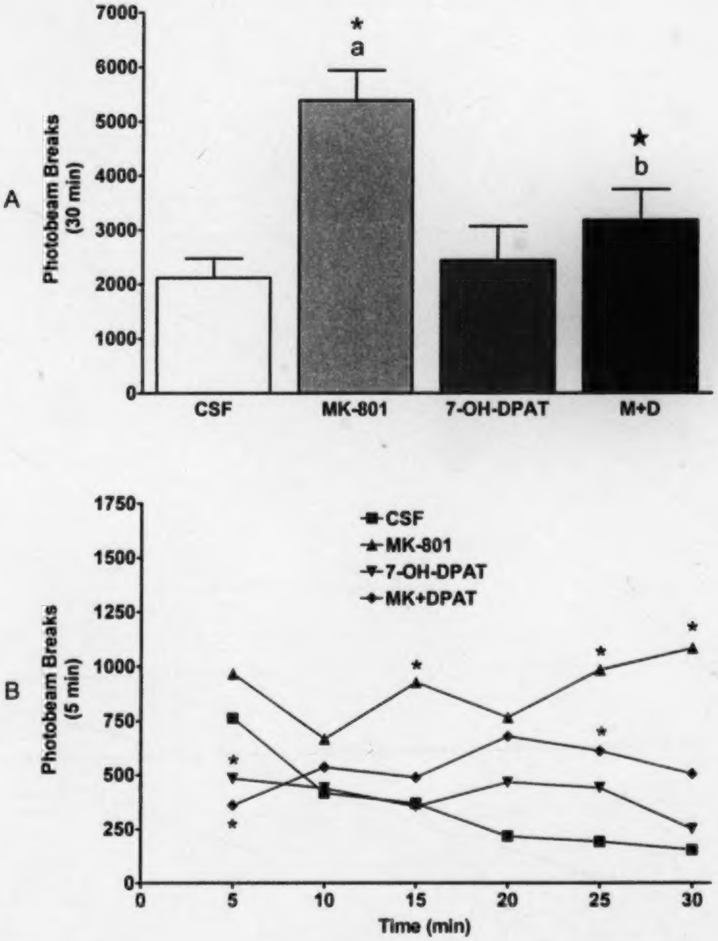

Interaural 11.20 mm
Interaural 10.70 mm
Interaural 10.60 mm
Interaural 10.20 mm

Figure 3.9. Effects of intra-NAS MK-801 (0.66 μg) and 7-OH-DPAT (5.0 μg) on total horizontal activity (A) (mean ± SEM; n=7). Significant main effect (*) or interaction (★) at P<0.05. Simple effects of MK-801 (a) and 7-OH-DPAT (b). The local time course data of horizontal activity (B) are shown as means (SEM omitted for clarity). Significant difference (*) from control at P<0.05. M+D refers to the combination of MK-801 and 7-OH-DPAT.



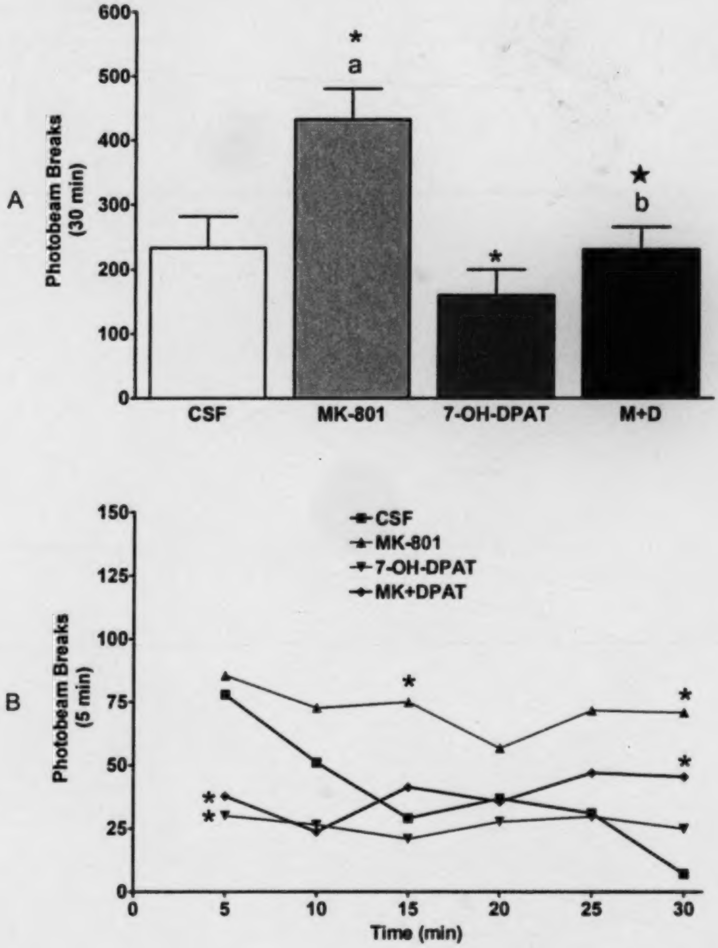
Consecutive activity

Three-way repeated measures ANOVA revealed significant main effects of MK-801 (F(1,6)=15.955, P=0.007) and 7-OH-DPAT (F(1,6)=20.102, P=0.004), and a significant MK-801 x 7-OH-DPAT interaction on consecutive activity (F(1,6)=6.762, P=0.041) (Figure 3.10(A)). There was no effect of time (F(2.034, 12.204)=1.355, P=0.295), nor significant MK-801 x time interaction (F(2.548, 15.289)=1.908, P=0.176), but there was a significant 7-OH-DPAT x time interaction (F(2.507, 15.041)=4.023, P=0.033). There was also no significant MK-801 x 7-OH-DPAT x time interaction (F(2.259, 13.552)=0.283, P=0.783).

Further analysis showed that MK-801 alone significantly increased activity (t(6)=5.100, P=0.002) but was ineffective in the presence of 7-OH-DPAT (t(6)=1.593, P=0.162). In contrast, 7-OH-DPAT alone was without effect (t(6)=1.977, P=0.095) but decreased activity in the presence of MK-801 (t(6)=4.851, P=0.003).

Analysis of local time course (Figure 3.10(B)) revealed that that MK-801 alone significantly increased activity at 15 and 30 minutes; 7-OH-DPAT alone significantly decreased activity during the first 5 minutes; and co-administration of MK-801 and 7-OH-DPAT initially significantly decreased activity during the first 5 minutes then increased activity at 30 minutes relative to CSF-treated control animals (Newman-Keuls P<0.05).

Figure 3.10. Effects of intra-NAS MK-801 (0.66 μg) and 7-OH-DPAT 5.0 μg) on total consecutive activity (A) (mean ± SEM; n=7). Significant main effect (*) or interaction (★) at P<0.05. Simple effects of MK-801 (a) and 7-OH-DPAT (b). The local time course data of consecutive activity (B) are shown as means (SEM omitted for clarity). Significant difference (*) from control at P<0.05. M+D refers to the combination of MK-801 and 7-OH-DPAT.



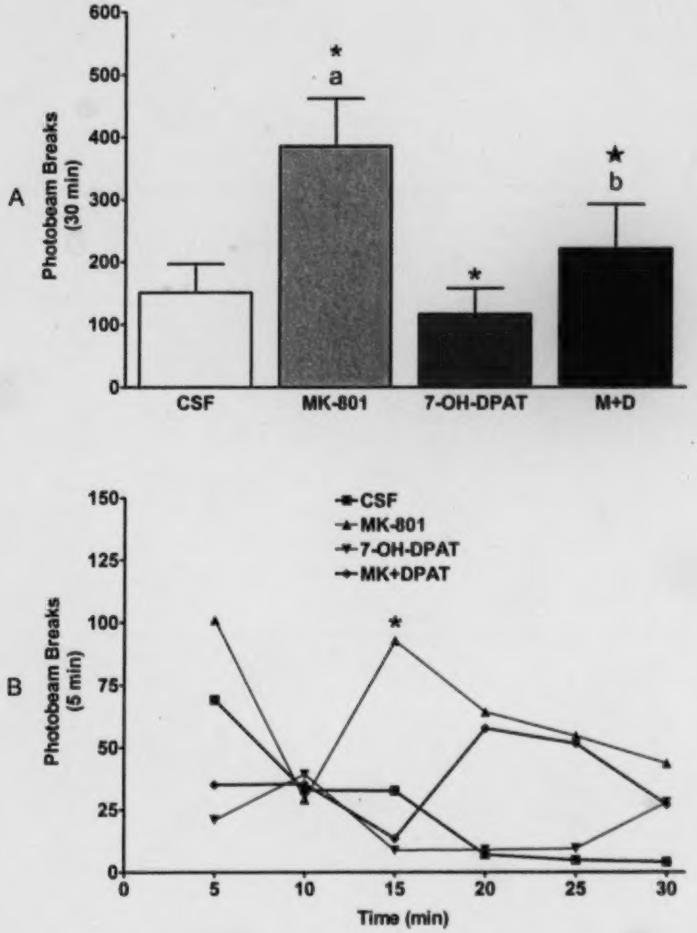
Vertical activity

Three-way repeated measures ANOVA revealed significant main effects of MK-801 (F(1,6)=15.987, P=0.007) and 7-OH-DPAT (F(1,6)=11.542, P=0.015), and a significant MK-801 x 7-OH-DPAT interaction on vertical activity (F(1,6)=21.866, P=0.003) (Figure 3.11(A)). There was no effect of time (F(1.973, 11.835)=3.016, P=0.088), but significant MK-801 x time and 7-OH-DPAT x time interactions (F(2.506, 15.037)=4.353, P=0.026 and F(2.937, 17.620)=5.882, P=0.006 respectively) without significant MK-801 x 7-OH-DPAT x time interaction (F(2.406, 14.436)=0.926, P=0.435).

Further analysis showed that MK-801 alone significantly increased activity (t(6)=4.840, P=0.003) but was ineffective in the presence of 7-OH-DPAT (t(6)=2.588, P=0.041 [Bonferroni α=0.025]). In contrast, 7-OH-DPAT alone was without effect (t(6)=1.404, P=0.210) but decreased activity in the presence of MK-801 (t(6)=4.261, P=0.005).

Analysis of local time course (Figure 3.11(B)) revealed that MK-801 alone significantly increased activity at 15 minutes relative to CSF-treated control animals (Newman-Keuls P<0.05).

Figure 3.11. Effects of intra-NAS MK-801 (0.66 μg) and 7-OH-DPAT (5.0 μg) on total vertical activity (A) (mean ± SEM; n=7). Significant main effect (*) or interaction (★) at P<0.05. Simple effects of MK-801 (a) and 7-OH-DPAT (b). The local time course data of vertical activity (B) are shown as means (SEM omitted for clarity). Significant difference (*) from control at P<0.05. M+D refers to the combination of MK-801 and 7-OH-DPAT.



Discussion

The main findings of this chapter are listed below. Each finding will be briefly discussed in turn in this chapter. Discussion that relates the separate studies to each other is reserved for Chapter 7 (General Discussion).

- MK-801 (0.13 mg kg^{-1} or 0.66 μg intra-NAS shell) induced hyperactivity (Experiments 1, 2, and 3).
- Apomorphine (0.05 mg kg^{-1}) induced hypoactivity (Experiment 1).
- 7-OH-DPAT (0.03 mg kg^{-1}) induced hypoactivity (Experiment 2).
- Effects of systemic MK-801 and apomorphine did not interact – effects of these compounds were additive (Experiment 1).
- Effects of systemic MK-801 and 7-OH-DPAT did interact. 7-OH-DPAT (0.03 mg kg^{-1}) blocked locomotor stimulation induced by MK-801 (0.03-0.13 mg kg^{-1}) (Experiment 2).
- Effects of intra-NAS MK-801 and 7-OH-DPAT did interact. Intra-NAS shell 7-OH-DPAT (5.0 μg) blocked locomotor stimulation induced by intra-NAS shell MK-801 (0.66 μg) (Experiment 3).

MK-801 (0.13 mg kg^{-1} or 0.66 μg intra-NAS shell) induced hyperactivity

MK-801 dose-dependently induced hyperactivity, in accord with several prior reports (e.g., Ford et al. 1989; Carlsson and Carlsson 1989; Maj et al. 1991; Löscher and Hönack 1992; Bubser et al. 1992; Willins et al. 1993; Starr and Starr

1994; Amalric et al. 1994; Ouagazzal et al. 1994; Vanover 1997; Adriani et al. 1998; Vanover 1998; Ninan and Kulkarni 1999). This effect may be due to activation of the mesolimbic DA system, as MK-801 activates VTA DA neurons (French and Ceci 1990; French et al. 1993) and increases DA turnover in the NAS (Bubser et al. 1992; Bristow et al. 1993; Mathé et al. 1998; Kretschmer 1999; Duan et al. 2005). MK-801 effects may also be DA-dependent, as this stimulant effect is blocked by the D_2-like DA receptor antagonists raclopride, YM 09151-2, sulpiride, haloperidol, or eticlopride, and may or may not be blocked by the D_1-like DA receptor antagonist SCH 23390 (Dall'Olio et al. 1992; Hoffman 1992; Ouagazzal et al. 1993; Mele et al. 1996; Adriani et al. 1998). More recently, Leriche et al. (2004) have shown that hyperactivity induced by MK-801 was reversed by D_3 DA receptor ligands, including BP 897 and nafadotride. However, there is contradictory evidence for the role of DA in MK-801 effects: MK-801 may not alter DA release in the NAS (Liljequist et al. 1991; Gandolfi et al. 1992; Druhan et al. 1996; Pierce et al. 1997), and stimulant effects of MK-801 have been described in monoamine-depleted mice and DA-deficient mice (Carlsson and Carlsson 1989; Svensson et al. 1992; Gossel et al. 1995; Svensson et al. 1995; Chartoff et al. 2005).

Consistent with systemic drug effects, intra-NAS shell MK-801 also significantly increased spontaneous locomotor activity, in accord with previous reports (Layer et al. 1991; Raffa et al. 1989; Svensson et al. 1994a; Svensson et al. 1995; Al-Khatib et al. 1995; Ouagazzal and Amalric 1995; Narayanan et al.

1996; Mele et al. 1998; Frantz and Van Hartesveldt 1999; De Leonibus et al. 2001).

Apomorphine (0.05 mg kg^{-1}) induced hypoactivity

Low dose apomorphine also induced hypoactivity, consistent with many prior studies (e.g., Di Chiara et al. 1976; Strömbom 1975; Sumners et al. 1981; Svensson et al. 1994b; Dziedzicka-Wasylewska and Rogóż 1997; Carey et al. 2004) but, in contrast to 7-OH-DPAT, this inhibitory effect has been attributed to the stimulation of presynaptic D_2-like DA receptors (Strömbom 1975; Strömbom 1976; Di Chiara et al. 1976; Strömbom 1977; Aghajanian and Bunney 1977; Zetterström and Ungerstedt 1984; Yarbrough et al. 1984; Ståhle and Ungerstedt 1984; Gessa et al. 1985; Cuomo et al. 1986; Ståhle and Ungerstedt 1986; Imperato et al. 1988; Carey et al. 2004).

7-OH-DPAT (0.03 mg kg^{-1}) induced hypoactivity

The locomotor suppressant effect of 7-OH-DPAT is also consistent with prior reports (Daly and Waddington 1993; Svensson et al. 1994b,c; Ahlenius and Salmi 1994; Storey et al. 1995; Ferrari and Giuliani 1995; Depoortere et al. 1996; Dall'Olio et al. 1997; Rogóż and Dziedzicka-Wasylewska 2000; Biondo 2002). This hypoactivity has been attributed to stimulation of "behaviourally-inhibitory" postsynaptic D_3 DA receptors (Daly and Waddington 1993; Waters et al. 1993; De Boer et al. 1997).

Effects of systemic MK-801 and apomorphine were additive

To the best of our knowledge, there are currently no studies that have investigated the interaction between NMDA receptor blockade and behavioural effects of low dose apomorphine. Most studies that have used apomorphine in this context employed higher doses that also stimulate postsynaptic DA receptors (Strömbom 1976). The current study is the first to demonstrate that the stimulatory effect of MK-801 and the inhibitory effect of low dose apomorphine on spontaneous locomotor activity are additive; there is no interaction between NMDA receptor blockade and a dose of apomorphine that presumably stimulates D_2-like DA autoreceptors. Therefore, locomotor stimulation induced by MK-801 may be independent of DA release in the NAS.

Systemic 7-OH-DPAT (0.03 mg kg^{-1}) blocked locomotor stimulation induced by MK-801 (0.03-0.13 mg kg^{-1})

In this study, there were significant MK-801 x 7-OH-DPAT interactions; 7-OH-DPAT (0.03 mg kg^{-1}) blocked the stimulatory effect of MK-801. This result is intriguing as Dall'Olio et al. (1997) showed that the $D_{2/3}$ DA agonists 7-OH-DPAT (0.005-0.08 mg kg^{-1}) and quinpirole (0.0125-0.050 mg kg^{-1}) may induce hypoactivity; while 7-OH-DPAT effects were blocked by MK-801 (0.06 mg kg^{-1}), quinpirole hypoactivity was unchanged. Consistent with these findings, our results also indicate that effects of MK-801 may interact with postsynaptic D_3 DA receptors coupled to decreased locomotor activity.

Intra-NAS shell 7-OH-DPAT (5.0 μg) was ineffective alone and blocked locomotor stimulation induced by intra-NAS shell MK-801 (0.66 μg)

In this study, there were also significant MK-801 x 7-OH-DPAT interactions; in the NAS, 7-OH-DPAT blocked the stimulatory effect of MK-801.

Intra-NAS 7-OH-DPAT alone was ineffective, in contrast to previous reports showing decreased activity (Kling-Peterson 1995a; Gilbert and Cooper 1995; Meyer 1996; Choi et al. 2000; Ouagazzal and Creese 2000). This discrepancy may be related to the lower number of subjects in the current study (n=7 vs. n=10-12 of prior studies), or the duration of testing (30 vs. 60 minutes). Despite this inconsistency, it is clear that intra-NAS shell 7-OH-DPAT blocked hyperactivity induced by MK-801. This finding concurs with the interaction of systemic effects described above and suggests that stimulation of $D_{2/3}$ DA receptors in the NAS shell subregion abolishes the stimulatory effect of NMDA receptor blockade. This finding contrasts with previous work that showed intra-NAS shell administration of 7-OH-DPAT induced hypoactivity that was potentiated by AMPA/kainate receptor antagonists CNQX or NBQX (Choi et al. 2000). The current results suggest that this type of relationship is not observed when NMDA receptor antagonists are used in place of AMPA receptor antagonists.

Conclusion

There was no interaction between the stimulant effect of MK-801 and stimulation of presynaptic D_2-like DA receptors by low dose apomorphine; effects of MK-801 and apomorphine were additive. In contrast, there was significant interaction between MK-801 and 7-OH-DPAT. 7-OH-DPAT induced hypoactivity via stimulation of D_3 DA receptors, likely located postsynaptically in the NAS, and blocked hyperactivity induced by systemic or intra-NAS shell MK-801. These findings suggest that increased arousal or locomotor stimulation induced by NMDA receptor blockade may be reversed by simultaneous stimulation of postsynaptic D_3 DA receptors, but not presynaptic D_2-like DA receptors. The next chapter will explore the effects of these compounds using a measurement of primary reinforcement: brain stimulation reward.

Chapter 4. Independence of NMDA receptor blockade and D_2-like DA receptor stimulation in the context of brain stimulation reward

(A version of this chapter has been accepted for publication in *Psychopharmacology* – see Clements & Greenshaw 2005b)

Introduction

Brain stimulation reward (BSR) is a paradigm in which a response performed by a laboratory animal (e.g., a lever-press) is reinforced by the contingent delivery of electrical stimulation to specific brain areas. The frequency of electrical stimulation that produces half-maximal responding is sensitive to changes in reinforcement induced by drugs, such as drugs of abuse (Wise 1996). Studies using DA receptor-related compounds to alter BSR in rats have contributed significantly to our understanding of the role of DA in disorders of motivation. This chapter has employed BSR to explore interactions between DA and Glu in the mesolimbic system.

Although there is some evidence that contrasts with a simple direct role of DA in mediating processes underlying motivation and reward (Garris et al. 1999), BSR generated by VTA stimulation increases DA release in the NAS; in addition, activation of D_2-like DA receptors in the NAS may play a role in mediating the rewarding and/or motivating effects of VTA stimulation (Phillips et al. 1989; Fiorino et al. 1993). The NAS is of primary interest in the context of BSR, as DA-containing cell bodies of the VTA project to the NAS, as do Glu projections from the prefrontal cortex (Sesack and Pickel 1992; Pennartz et al. 1994). The NAS, in addition to the VTA, islands of Calleja, and olfactory tubercle, contains a relatively high density of D_3 DA receptors (Lévesque et al. 1992; Diaz et al. 2000).

Studies using systemic administration of the $D_{2/3}$ DA receptor agonist 7-OH-DPAT have failed to reveal consistent effects on BSR (Gilbert et al. 1995; Depoortere et al. 1996; Hatcher and Hagan 1998; Baldo et al. 1999a; Depoortere et al. 1999). As 7-OH-DPAT is the most selective agonist currently available to stimulate D_3 DA receptors, the effects of this compound on VTA BSR following systemic administration have been investigated in the present study. As the behavioural effects of doses of 7-OH-DPAT used in the present study have been attributed to stimulation of postsynaptic D_3 DA receptors (Waters et al. 1993; Svensson et al. 1994c), we have also employed a low dose of the DA agonist apomorphine. As described in Chapter 1 (see page 24), low doses of apomorphine show moderate $D_{2/3}$ DA receptor selectivity in vivo, and its behavioural effects have been attributed to stimulation of presynaptic D_2-like DA autoreceptors (Strömbom 1975; Strömbom 1976; Di Chiara et al. 1976; Strömbom 1977; Aghajanian and Bunney 1977; Zetterström and Ungerstedt 1984; Yarbrough et al. 1984; Ståhle and Ungerstedt 1984; Gessa et al. 1985; Cuomo et al. 1986; Ståhle and Ungerstedt 1986; Imperato et al. 1988; Carey et al. 2004).

The purposes of the current investigation were to use BSR rate-frequency analysis to assess: 1) effects of the NMDA receptor antagonist MK-801; 2) effects of the $D_{2/3}$ DA receptor agonist 7-OH-DPAT; 3) effects of presumably D_2-like DA receptor stimulation by low dose apomorphine; 4) to characterize the interaction between NMDA receptor blockade and $D_{2/3}$ DA receptor stimulation using 7-OH-DPAT; and 5) to characterize the interaction between NMDA receptor blockade and presumably presynaptic D_2-like DA receptor stimulation using low dose apomorphine.

Based on the results of Choi (2000), who demonstrated that AMPA/kainate receptor blockade and 7-OH-DPAT alone were ineffective but combined increased VTA BSR rate-frequency thresholds (i.e., decrease reward), it was hypothesized that NMDA receptor blockade may also show interaction with the effects of D_2-like DA receptor stimulation.

Hypotheses

The hypotheses for experiments described in this chapter were as follows:

1. Systemic MK-801 alone would lower BSR rate-frequency thresholds.
2. Systemic 7-OH-DPAT or apomorphine would increase BSR rate-frequency thresholds.
3. MK-801 combined with 7-OH-DPAT or apomorphine may increase BSR rate-frequency thresholds.

Materials and methods

Animals

Male Sprague-Dawley rats (200-250 g; Health Sciences Laboratory Animal Services, University of Alberta) were housed individually in a temperature- (21±1°C) and humidity-controlled environment with a 12-hour light/dark cycle (lights on 0700-1900 hours). Food (LabDiet 5001 Rodent Diet, PMI Nutrition International Inc., Brentwood, MO, USA) and water were freely available. Separate groups of animals were used for each experiment, and testing always occurred between 0900-1700 hours. The care and use of animals was approved by the Health Sciences Animal Policy and Welfare Committee and the University Animal Policy and Welfare Committee at the University of Alberta, and complied with the "Guide to the Care and Use of Experimental Animals" (Volume 1, 2nd Edition, 1993; Volume 2, 1984) published by the Canadian Council on Animal Care (CCAC).

Drugs

(+)-MK-801 maleate [dizocilpine; (5S, 10R)-(+)-5-methyl-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5,10-imine maleate], 7-OH-DPAT ((±)-7-hydroxy-2-dipropylaminotetralin) hydrobromide, and apomorphine hydrochloride hemi-hydrate were obtained from Sigma RBI (Oakville, ON, CA). Compounds were dissolved in isotonic saline (Fisher Scientific Ltd., Nepean, ON, CA) and drug solutions were made daily (pH 5.0-7.0) according to the protocol in Appendix A.

Doses are expressed as free bases. In experiments 4 and 5, MK-801 (0.03, 0.06, or 0.13 mg kg^{-1}) was given IP 30 minutes prior while 7-OH-DPAT (experiment 4; 0.03 mg kg^{-1}) or apomorphine (experiment 5; 0.05 mg kg^{-1}) was given SC 15 or 10 minutes prior respectively. All compounds were given in a volume of 1.0 mL kg^{-1}. The dose of 7-OH-DPAT used in these experiments was based upon a prior dose-response analysis of spontaneous exploratory activity in this laboratory (Biondo 2002). The dose of apomorphine demonstrates behavioural effects similar to 0.03 mg kg^{-1} 7-OH-DPAT (i.e., decreases spontaneous activity) but, unlike this dose of 7-OH-DPAT, stimulates DA autoreceptors (see Chapter 1).

Stereotaxic surgery

A standard operating procedure for central implantation is provided in Appendix C. Briefly, under aseptic conditions, animals weighing between 300-350 g were anaesthetized using Halothane (2-bromo-2-chloro-1,1,1-trifluoroethane; Halocarbon Laboratories, River Edge, NJ, USA) and placed in a Kopf stereotaxic instrument (Kopf Instruments, Tujunga, CA, USA). Animals were implanted with a unilateral monopolar stimulating electrode (E363/2, Plastics One Inc., Roanoke, VA, USA; diameter 200 µm) directed at the VTA. Stereotaxic coordinates were interpolated from the target site (defined by Paxinos and Watson 1986) using angular placements to avoid damage to blood sinuses and cerebral ventricles (Greenshaw 1997b). The VTA coordinates are relative to inter-aural zero with the incisor bar set 2.4 mm below the inter-aural line: VTA AP

+3.2 mm, LM +0.6 mm, DV +2.3 mm, 20° lateral and 20° anterior to the sagittal and coronal planes respectively. In addition, an indifferent electrode (E363/0, Plastics One Inc., Roanoke, VA, USA) with silver wire was fixed to the frontal bone. The electrodes, through an electrode pedestal (MS363, Plastics One Inc., Roanoke, VA, USA), were fixed to the skull using stainless steel screws (Lomat Watch Co., Montréal, QC, CA) and dental acrylic (Caulk/Dentsply, Milford, DE, USA). Testing began at least one week after surgery.

Apparatus

Experiments were carried out in ventilated operant test chambers (24 cm L x 30 cm W x 29 cm H) each equipped with a sound-attenuating outer chamber (Coulbourn Instruments Ltd., Lehigh Valley, PA, USA). Gold track slip rings allowed free movement of the animal and were connected to constant current programmable stimulators (I. Halvorsen Systems Design, Phoenix, AZ, USA). Stimulation parameters and recording of behavioural responses were under computer control. Between pulses, the stimulating and ground electrodes were connected through a 10 kΩ resistor to cancel any effects of electrode polarization. Throughout the experiment, each animal was always exposed to the same apparatus that was cleaned thoroughly between animals using diluted ammonia-based window cleaner (1:6 in water) (No Name Glass Cleaner with Ammonia, Loblaw Companies Ltd., Toronto, ON).

Training and baseline procedures

Following recovery, each animal was placed in the apparatus and trained to lever-press for electrical stimulation of the VTA (cathodal pulse width 200 ms; frequency 100 Hz; train length 1 s). Initially, the animal's exploratory behaviour was shaped to determine a current intensity (80-400 μA) that maintained lever-pressing. Once established, daily training sessions on continuous reinforcement schedules allowed each animal to associate lever-pressing with brain stimulation. Once responding stabilized, animals underwent daily sessions designed to determine the stimulating current that produced half-maximal responding, while stimulation frequency was held constant at 100 Hz. This was determined using rate-intensity analysis that consisted of stepwise changes in current every 2 minutes while all other parameters remained constant. This regimen continued until the half-maximal current varied less than 10% for three consecutive days. For each animal, this current was used for all subsequent daily sessions. Animals then underwent daily sessions designed to determine the stimulation frequency (0-160 Hz) that produced half-maximal responding (rate-frequency threshold or M_{50}). One rate-frequency function was generated per day, using rate-frequency analysis consisting of stepwise changes in frequency while other parameters, including current, remained constant. Responding was initiated at 160 Hz and decreased stepwise each minute by 0.1 log Hz until 0 Hz (or until the animal stopped responding for the duration of a single frequency). Stimulation frequency was then reversed to increase stepwise each minute by 0.1 log Hz up to 160 Hz.

For each animal, the descending and ascending segments were collapsed to generate a single rate-frequency function; linear regression analysis was used to determine M_{50}. This regimen was continued until the M_{50} varied less than 10% across three consecutive days. The M_{50}, maximum response rate (RMAX), and total responses (TRES) were recorded during all subsequent daily sessions. At the beginning of each time bin for rate-intensity and rate-frequency analysis, each animal received three trains of stimulation (primes) that served as a discriminative stimulus to indicate that stimulation was available. This method of measuring rate-frequency thresholds provides an objective measure of reward sensitivity that is easily distinguished from nonspecific changes in behaviour, while the maximum response rate is a measure of response performance (Gallistel and Karras 1984; Greenshaw and Wishart 1987).

Experimental designs

Experiment 4 was divided into two parts: two groups of animals (n=9 per group) received a randomized counterbalanced sequence of four treatments, with two days of baseline rate-frequency testing between each treatment: SAL + SAL, SAL + MK-801 (0.03 mg kg^{-1} IP in experiment 4a or 0.13 mg kg^{-1} IP in experiment 4b), SAL + 7-OH-DPAT (0.03 mg kg^{-1} SC), and MK-801 + 7-OH-DPAT. In experiment 5, animals (n=11) received a randomized counterbalanced sequence of six treatments, with two days of baseline rate-frequency testing between each treatment: SAL + SAL, SAL + MK-801 (0.03 or 0.13 mg kg^{-1}

respectively), SAL + apomorphine (0.05 mg kg^{-1}), and each combination of MK-801 + apomorphine. Routes and timing of drug administration are described above under the heading *Drugs*. All experiments consisted of a repeated measures design to minimize the number of animals required.

Histology

Animals were deeply anaesthetized with sodium pentobarbital (Bimeda-MTC Animal Health Inc., Cambridge, ON, CA; Somnotol or Euthanyl) and perfused intracardially with 50 mL ice-cold isotonic saline (Fisher Scientific Ltd., Nepean, ON, CA) followed by 50 mL 10% w/v buffered formalin phosphate (Fisher Scientific Ltd., Nepean, ON, CA). Following decapitation, brains were removed and stored at room temperature in 10% w/v buffered formalin phosphate for 4-6 hours, then stored in 30% sucrose/10 mM PBS buffer for at least 24 hours. Brains were then flash-frozen by immersion in isopentane (2-methylbutane; Sigma-Aldrich Co., Oakville, ON, CA) chilled on solid carbon dioxide (Praxair Canada Inc., Edmonton, AB, CA). Brains were briefly dried and stored at –80°C until sectioning. Electrode placements were verified by inspection of 40 µm coronal brain sections. This histology protocol is described further in Appendix D. Only animals with correct electrode placements were included in data analyses. A representative photomicrograph of an electrode tip in the VTA is shown in Appendix F.

Statistical analyses

Data are presented as % of baseline response (M_{50}, RMAX, or TRES) defined as the mean of data collected during the drug-free days prior to each treatment (as noted above), and all analyses were completed using these % baseline data. In experiments 4a and 4b, effects of MK-801 and 7-OH-DPAT on each measure were assessed by 2 x 2 repeated measures ANOVA (MK-801 x 7-OH-DPAT). In experiment 5, the effects of MK-801 and apomorphine were assessed by 3 x 2 repeated measures ANOVA (MK-801 x apomorphine). In the absence of MK-801 x 7-OH-DPAT or MK-801 x apomorphine interaction, the main effect of MK-801 was explored using a one-way repeated measures ANOVA followed by Newman-Keuls post-hoc tests to compare each dose of MK-801 to vehicle. Statistical analyses (α=0.05) were completed using statistical software (SPSS Inc., Chicago, IL, USA).

For each repeated measures experiment, Greenhouse-Geisser adjusted degrees of freedom have been used; because of this, degrees of freedom values may appear as non-integers.

Rate-frequency regression curves

For illustrative purposes, group-averaged rate-frequency curves were derived by converting response rate per frequency to a percentage of maximum response rate for each animal, and then averaging the percentages for each frequency by treatment group. In accordance with prior work from this laboratory

(Choi 2000), non-linear regression curves were generated by Prism software (GraphPad Software, Inc., San Diego, CA, USA) using the sigmoidal dose-response equation with a variable slope:

$$y = \text{min} + \frac{\text{max} - \text{min}}{1 + 10^{(\text{logEC50} - x)\text{Hillslope}}}$$

It is noteworthy that, as described above, M_{50} was calculated for individual animals each day using linear regression; illustrative group-averaged rate-frequency curves derived from non-linear regression are used for a convenient visual summary of performance. While regression results are usually concordant, discrepancies may arise due to differences in regression equation parameters.

Results

Experiment 4a: Effects of MK-801 (0.03 mg kg^{-1}) and 7-OH-DPAT on VTA BSR

Histological locations of VTA electrode placements for this experiment are shown in Figure 4.1.

The general pattern of results indicates that there was no interaction between the effects of MK-801 and 7-OH-DPAT on BSR on any measure; effects of MK-801 and 7-OH-DPAT were additive.

There were significant main effects of MK-801 (F(1,8)=6.365, P=0.036) and 7-OH-DPAT (F(1,8)=25.267, P=0.001) on M_{50}, but there was no significant MK-801 x 7-OH-DPAT interaction (F(1,8)=0.116, P=0.743) on this measure (Figure 4.2). There was a significant main effect of 7-OH-DPAT on RMAX (F(1,8)=5.472, P=0.047), but there was no main effect of MK-801 (F(1,8)=0.411, P=0.539) nor MK-801 x 7-OH-DPAT interaction (F(1,8)=0.585, P=0.466) on this measure (Figure 4.3). There were significant main effects of MK-801 (F(1,8)=7.263, P=0.027) and 7-OH-DPAT (F(1,8)=37.753, P<0.001) on TRES, but there was no significant MK-801 x 7-OH-DPAT interaction (F(1,8)=2.698, P=0.139) on this measure (Figure 4.4).

As shown in Figure 4.5, administration of MK-801 shifted the rate-frequency curve to the left, indicating increased reward, while 7-OH-DPAT or co-administration of MK-801 and 7-OH-DPAT shifted the rate-frequency curve to the right, indicating decreased reward.

Figure 4.1. Illustration of histological verification of electrode sites in the VTA. Numbers represent distances in the coronal plane from interaural zero according to the atlas of Paxinos and Watson (1986).

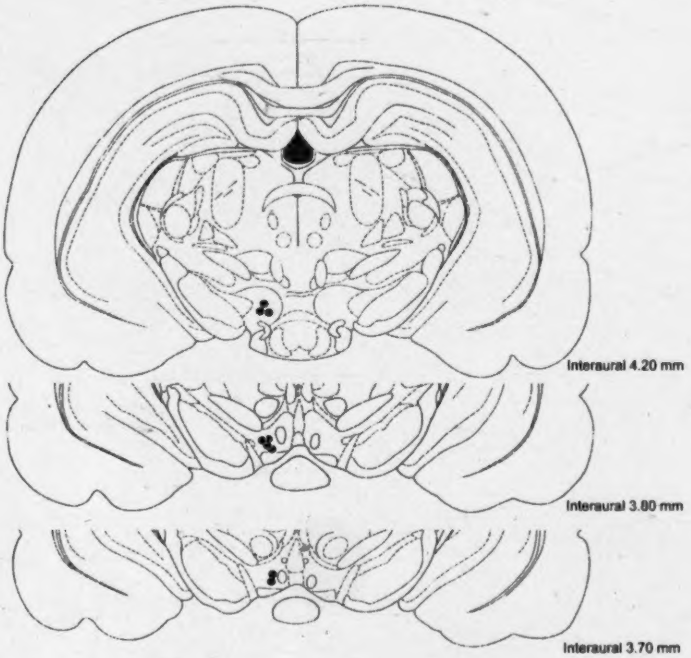

Interaural 4.20 mm
Interaural 3.80 mm
Interaural 3.70 mm

Figure 4.2. Effects of MK-801 (0.03 mg kg^{-1}) and 7-OH-DPAT (0.03 mg kg^{-1}) on BSR rate-frequency thresholds (M_{50}). Data are presented as mean ± SEM (n=9). Significant main effect (*) at $P<0.05$. M+D refers to the combination of MK-801 and 7-OH-DPAT.

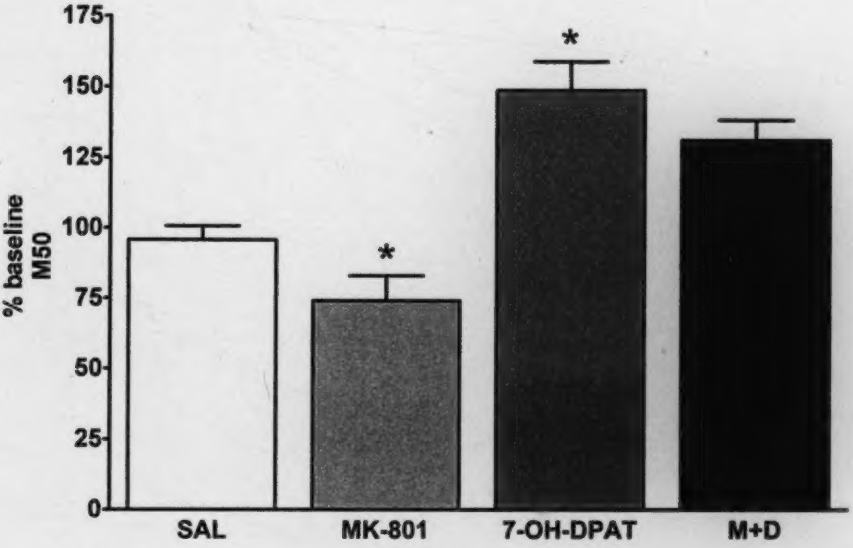


Figure 4.3. Effects of MK-801 (0.03 mg kg^{-1}) and 7-OH-DPAT (0.03 mg kg^{-1}) on BSR maximum response (RMAX). Data are presented as mean ± SEM (n=9). Significant main effect (*) at P<0.05. M+D refers to the combination of MK-801 and 7-OH-DPAT.

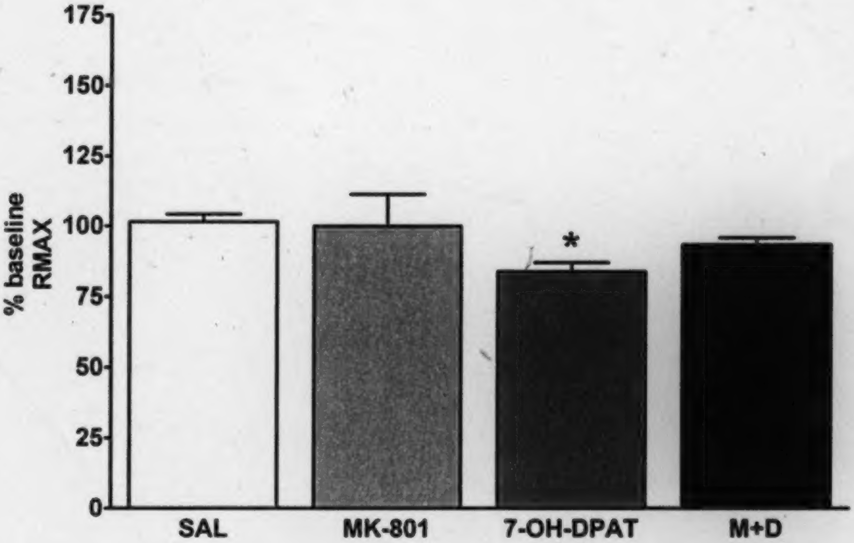


Figure 4.4. Effects of MK-801 (0.03 mg kg^{-1}) and 7-OH-DPAT (0.03 mg kg^{-1}) on BSR total responses (TRES). Data are presented as mean ± SEM (n=9). Significant main effect (*) at $P<0.05$. M+D refers to the combination of MK-801 and 7-OH-DPAT.

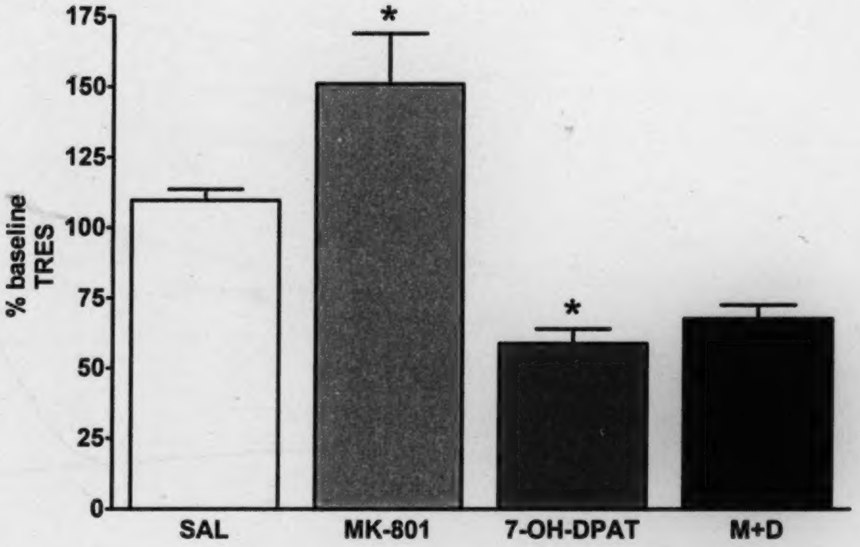
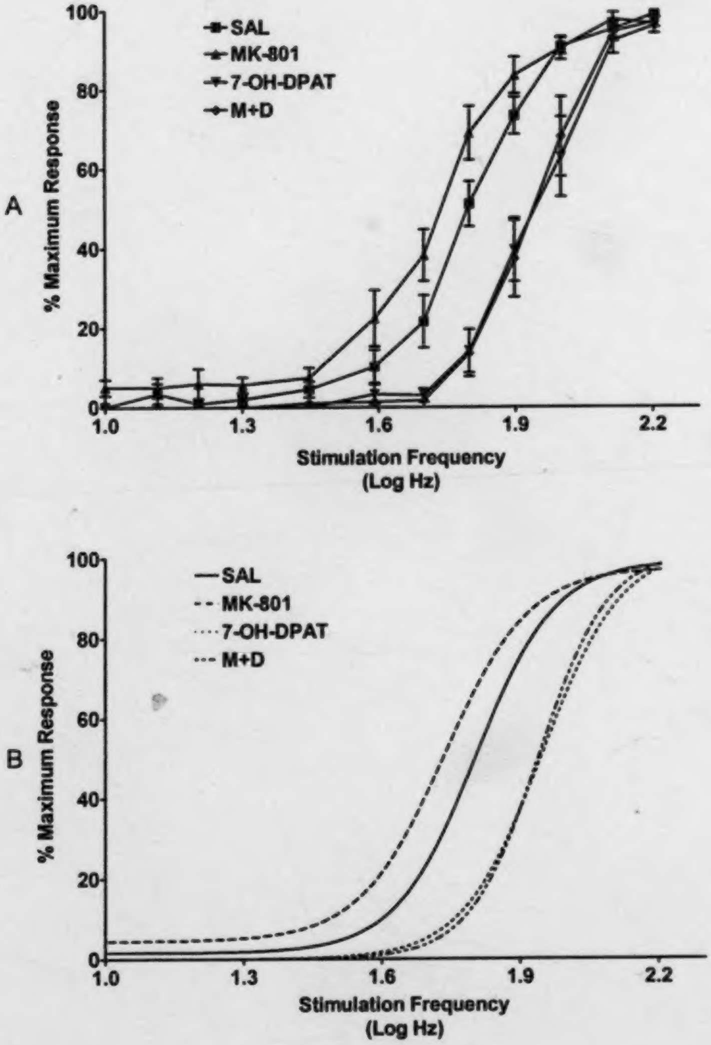


Figure 4.5. Rate-frequency data (A) and non-linear regression curves (B) for MK-801 (0.03 mg kg^{-1}) and 7-OH-DPAT (0.03 mg kg^{-1}). Data are presented as mean ± SEM (n=9). M+D refers to the combination of MK-801 and 7-OH-DPAT.



Experiment 4b: Effects of MK-801 (0.13 mg kg^{-1}) and 7-OH-DPAT on VTA BSR

Histological locations of VTA electrode placements for this experiment are shown in Figure 4.6.

The general pattern of results indicates that there was no interaction between the effects of MK-801 and 7-OH-DPAT on BSR on any measure; effects of MK-801 and 7-OH-DPAT were additive.

There were significant main effects of MK-801 (F(1,8)=21.250, P=0.002) and 7-OH-DPAT (F(1,8)=34.349, P<0.001) on M_{50}, but there was no significant MK-801 x 7-OH-DPAT interaction (F(1,8)=0.968, P=0.354) on this measure (Figure 4.7). There were no significant main effects of MK-801 (F(1,8)=4.924, P=0.057) or 7-OH-DPAT (F(1,8)=1.976, P=0.197) on RMAX, and there was no MK-801 x 7-OH-DPAT interaction (F(1,8)=0.454, P=0.519) on this measure (Figure 4.8). There were significant main effects of MK-801 (F(1,8)=13.099, P=0.007) and 7-OH-DPAT (F(1,8)=40.446, P<0.001) on TRES, but there was no significant MK-801 x 7-OH-DPAT interaction (F(1,8)=1.196, P=0.306) on this measure (Figure 4.9).

As shown in Figure 4.10, administration of MK-801 shifted the rate-frequency curve to the left, indicating increased reward, while 7-OH-DPAT shifted the rate-frequency curve to the right, indicating decreased reward.

Figure 4.6. Illustration of histological verification of electrode sites in the VTA. Numbers represent distances in the coronal plane from interaural zero according to the atlas of Paxinos and Watson (1986).

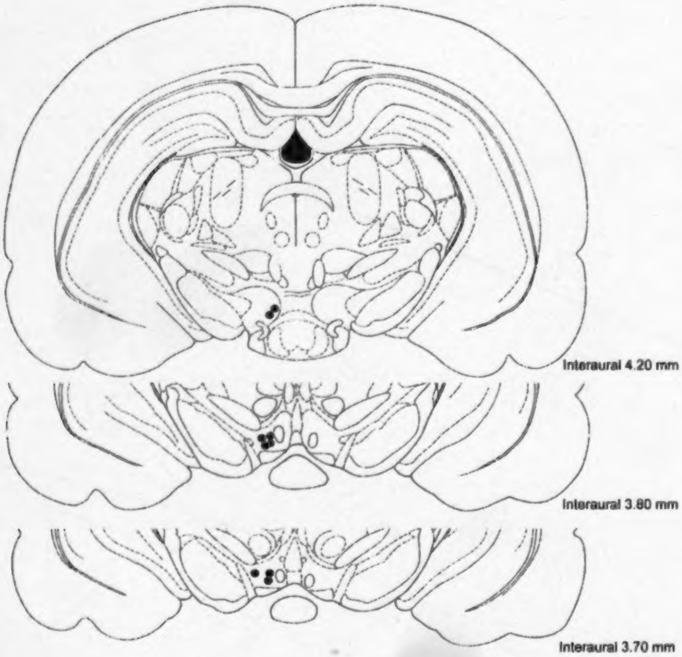

Interaural 4.20 mm
Interaural 3.80 mm
Interaural 3.70 mm

Figure 4.7. Effects of MK-801 (0.13 mg kg^{-1}) and 7-OH-DPAT (0.03 mg kg^{-1}) on BSR rate-frequency thresholds (M_{50}). Data are presented as mean ± SEM (n=9). Significant main effect (*) at $P<0.05$. M+D refers to the combination of MK-801 and 7-OH-DPAT.

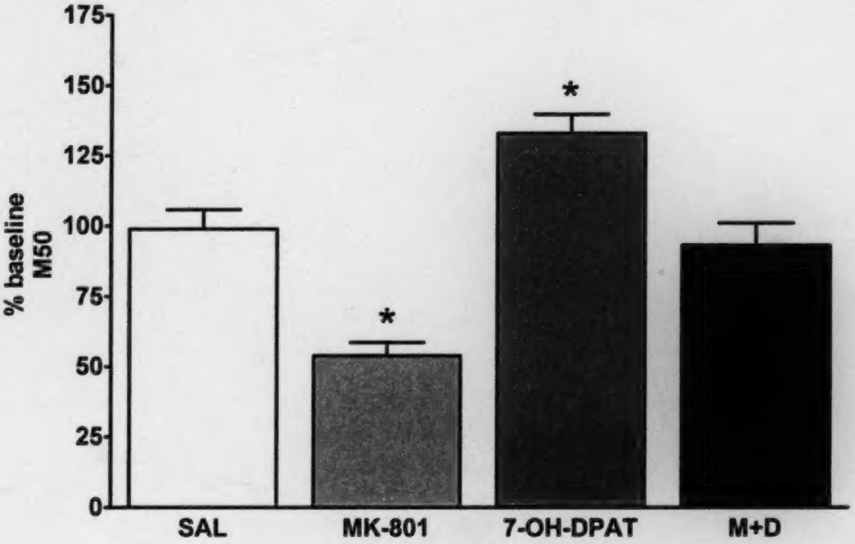


Figure 4.8. Effects of MK-801 (0.13 mg kg^{-1}) and 7-OH-DPAT (0.03 mg kg^{-1}) on BSR maximum response (RMAX). Data are presented as mean ± SEM (n=9). M+D refers to the combination of MK-801 and 7-OH-DPAT.

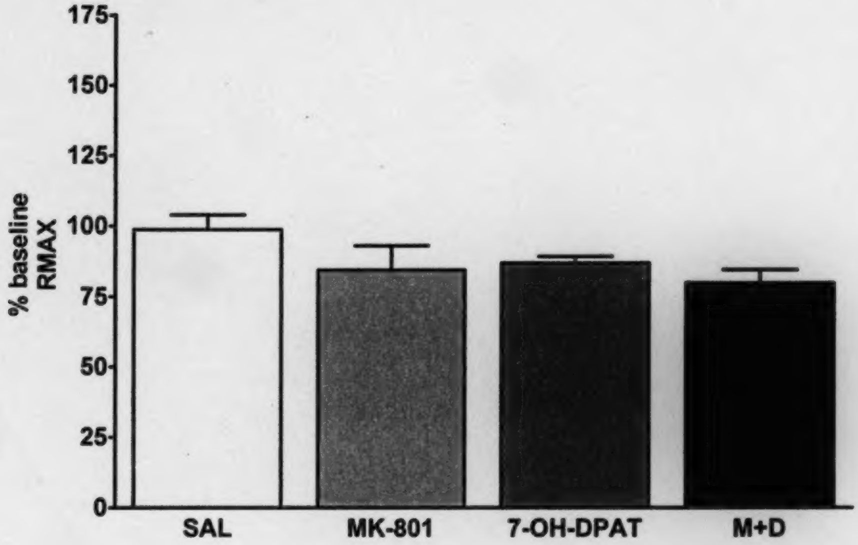


Figure 4.9. Effects of MK-801 (0.13 mg kg^{-1}) and 7-OH-DPAT (0.03 mg kg^{-1}) on BSR total responses (TRES). Data are presented as mean ± SEM (n=9). Significant main effect (*) at P<0.05. M+D refers to the combination of MK-801 and 7-OH-DPAT.

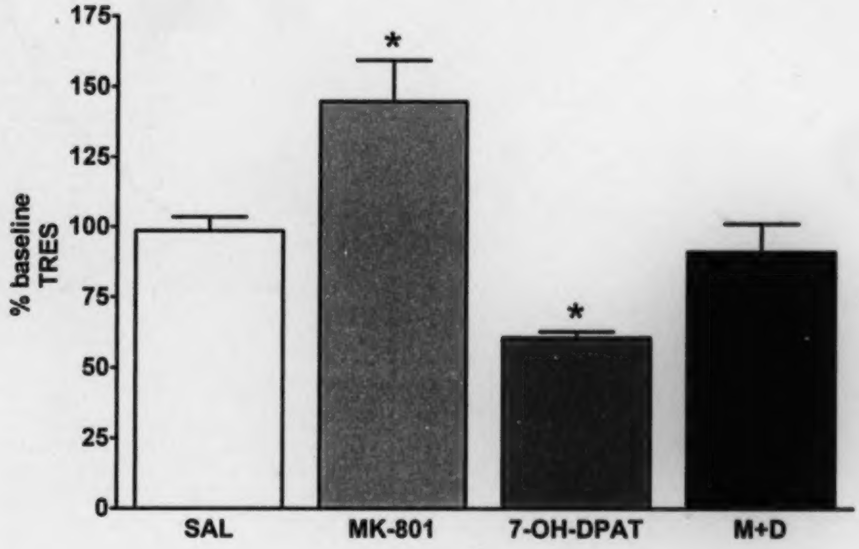
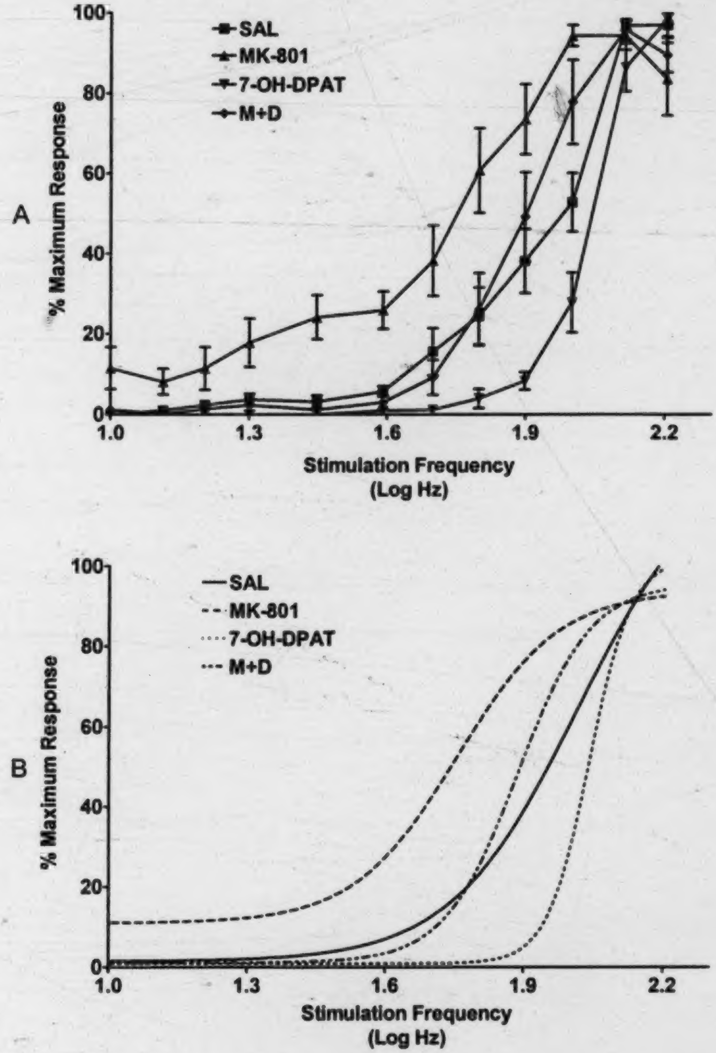


Figure 4.10. Rate-frequency data (A) and non-linear regression curves (B) for MK-801 (0.13 mg kg^{-1}) and 7-OH-DPAT (0.03 mg kg^{-1}). Data are presented as mean ± SEM (n=9). M+D refers to the combination of MK-801 and 7-OH-DPAT.



Experiment 5: Effects of systemic MK-801 and apomorphine on VTA BSR

Histological locations of VTA electrode placements for this experiment are shown in Figure 4.11.

The general pattern of results indicates that there was no interaction between the effects of MK-801 and apomorphine on BSR on any measure; effects of MK-801 and apomorphine were additive.

There were significant main effects of MK-801 ($F(1.786,17.855)=14.892$, $P<0.001$) and apomorphine ($F(1,10)=7.659$, $P=0.020$) on M_{50}, but there was no significant MK-801 x apomorphine interaction ($F(1.739,17.391)=0.270$, $P=0.737$) on this measure (Figure 4.12). Post-hoc tests revealed that MK-801 (0.13 mg kg^{-1}) significantly reduced M_{50} relative to vehicle (Figure 4.13) (Newman-Keuls $P<0.05$). There was a main effect of apomorphine ($F(1,10)=6.897$, $P=0.025$) on RMAX, but no main effect of MK-801 ($F(1.661, 16.608)=0.152$, $P=0.822$) nor MK-801 x apomorphine interaction ($F(1.289, 12.891)=0.989$, $P=0.362$) on this measure (Figure 4.14). There were significant main effects of MK-801 ($F(1.972,19.717)=18.615$, $P<0.001$) and apomorphine ($F(1,10)=38.429$, $P<0.001$) on TRES, but there was no significant MK-801 x apomorphine interaction ($F(1.549,15.494)=0.053$, $P=0.912$) on this measure (Figure 4.15). Post-hoc tests revealed that MK-801 (0.13 mg kg^{-1}) significantly increased TRES relative to vehicle (Figure 4.16) (Newman-Keuls $P<0.05$).

As shown in Figure 4.17, MK-801 shifted the rate-frequency curve to the left, indicating increased reward, while apomorphine had opposite effects.

Figure 4.11. Illustration of histological verification of electrode sites in the VTA. Numbers represent distances in the coronal plane from interaural zero according to the atlas of Paxinos and Watson (1986).

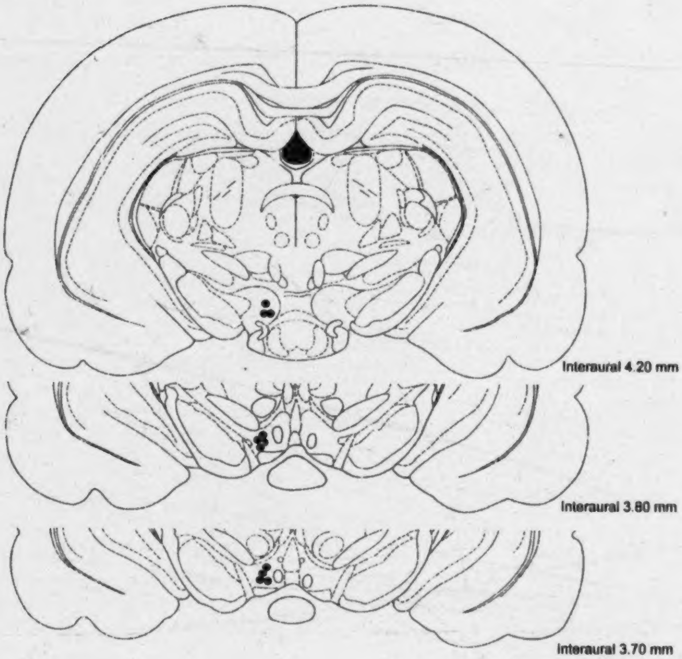

Interaural 4.20 mm
Interaural 3.80 mm
Interaural 3.70 mm

Figure 4.12. Effects of MK-801 and apomorphine (0.05 mg kg^{-1}) on BSR rate-frequency thresholds (M_{50}). Data are presented as mean ± SEM (n=11).

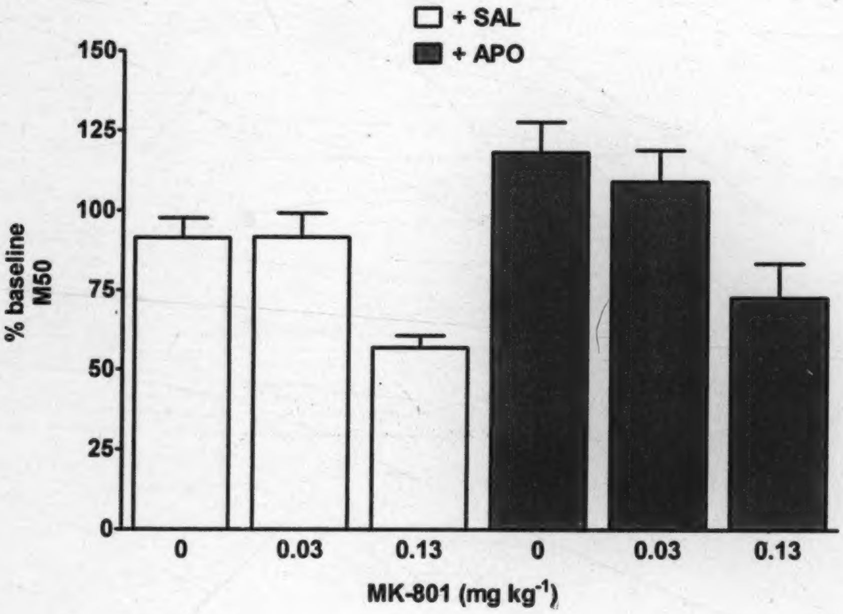


Figure 4.13. Further analysis of the main effect of MK-801 on BSR rate-frequency thresholds (M_{50}). Data are presented as mean ± SEM (n=22). Significant difference (*) from control at $P<0.05$.

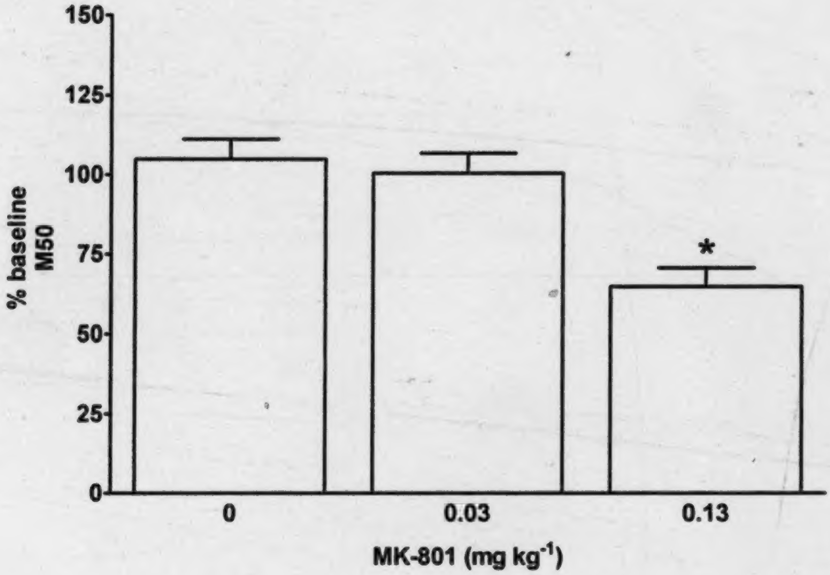


Figure 4.14. Effects of MK-801 and apomorphine (0.05 mg kg^{-1}) on BSR maximum response (RMAX). Data are presented as mean ± SEM (n=11).

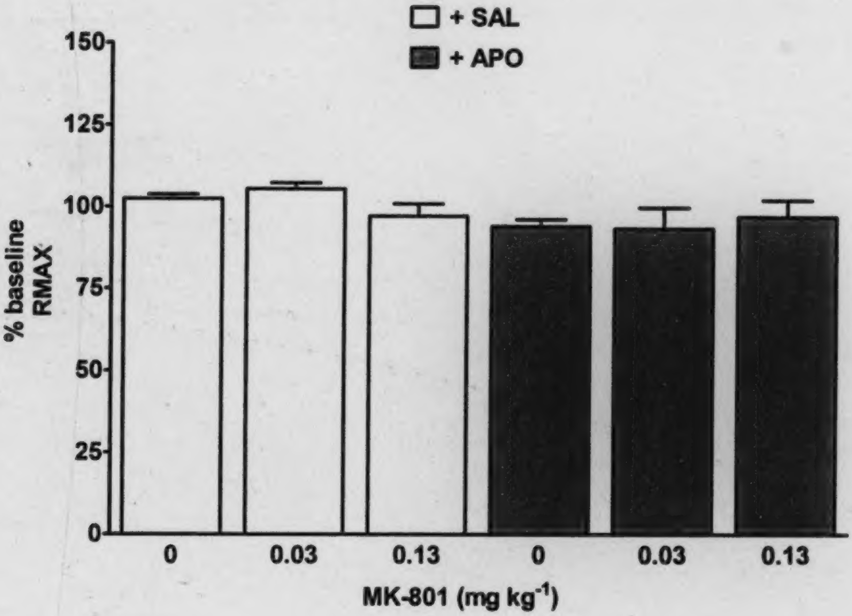


Figure 4.15. Effects of MK-801 and apomorphine (0.05 mg kg^{-1}) on BSR total responses (TRES). Data are presented as mean ± SEM (n=11).

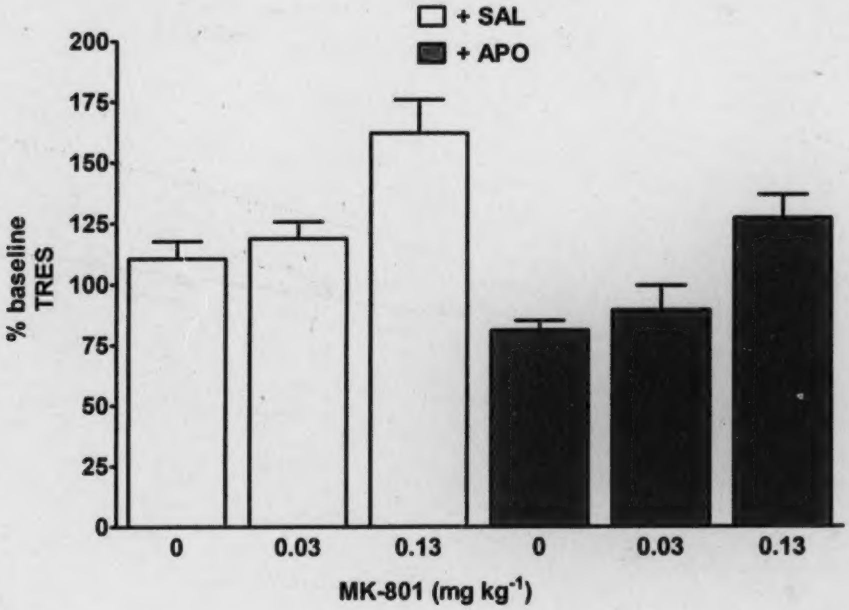


Figure 4.16. Further analysis of the main effect of MK-801 on BSR total responses (TRES). Data are presented as mean ± SEM (n=22). Significant difference (*) from control at P<0.05.

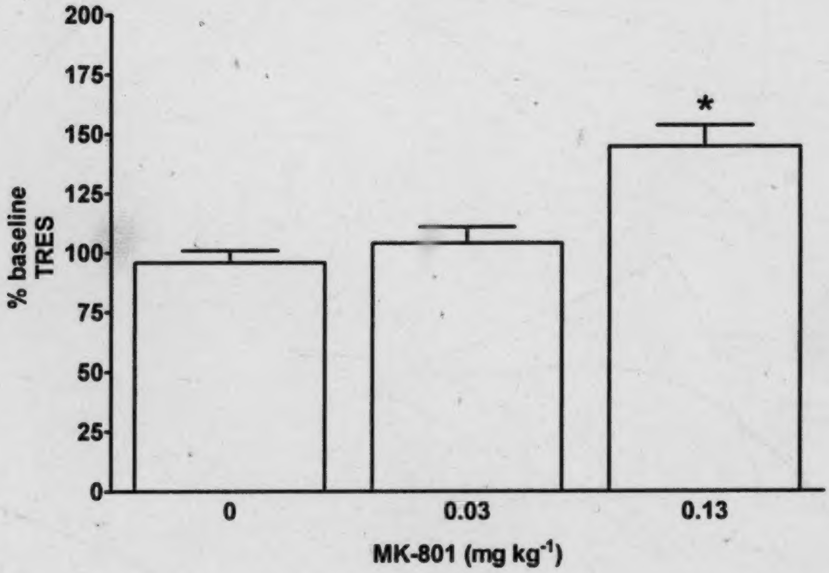
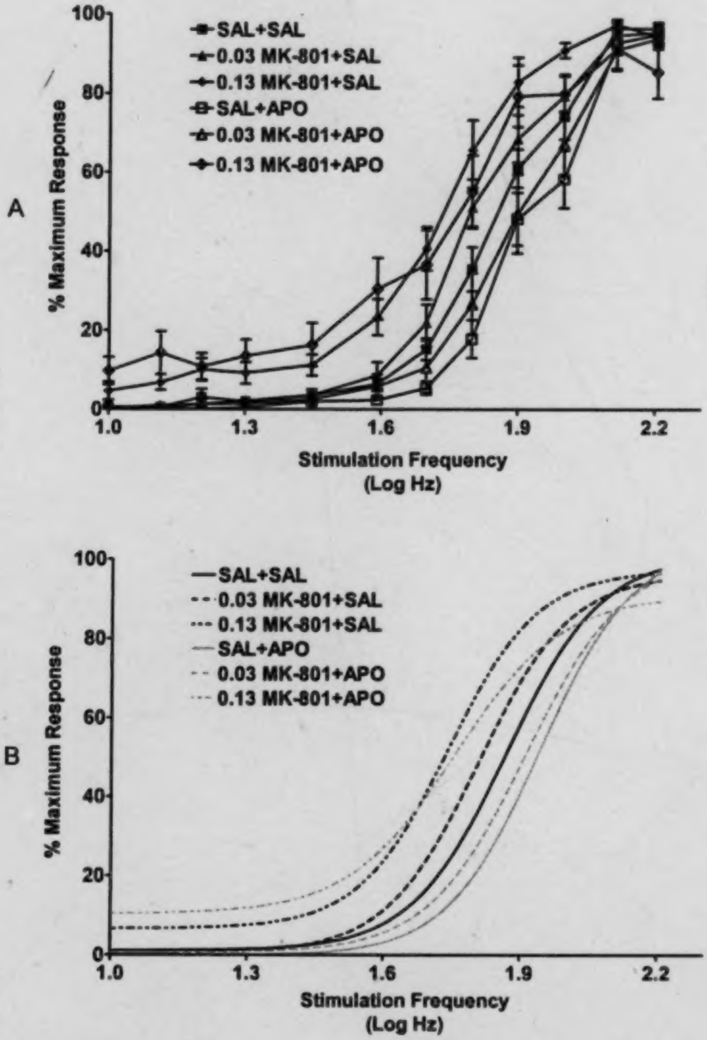


Figure 4.17. Rate-frequency data (A) and non-linear regression curves (B) for MK-801 and apomorphine (0.05 mg kg^{-1}). Data are presented as mean ± SEM (n=11).



Discussion

The main findings of this chapter are listed below. Each finding will be briefly discussed in turn in this chapter. Discussion that relates the separate studies to each other is reserved for Chapter 7 (General Discussion).

- MK-801 (0.03 mg kg^{-1} or 0.13 mg kg^{-1} IP 30 min prior) decreased BSR rate-frequency thresholds, indicating increased reward (Experiments 4 and 5).
- 7-OH-DPAT (0.03 mg kg^{-1} SC 15 min prior) increased BSR rate-frequency thresholds, indicating decreased reward (Experiment 4).
- Apomorphine (0.05 mg kg^{-1} SC 10 min prior) increased BSR rate-frequency thresholds, indicating decreased reward (Experiment 5).
- Effects of systemic administration of MK-801 and 7-OH-DPAT on BSR rate-frequency thresholds did not interact (Experiment 4).
- Effects of systemic administration of MK-801 and apomorphine on BSR rate-frequency thresholds did not interact (Experiment 5).

MK-801 (0.03 mg kg^{-1} or 0.13 mg kg^{-1} IP 30 min prior) decreased BSR rate-frequency thresholds, indicating increased reward

In the present study, MK-801 decreased rate-frequency thresholds for VTA BSR, in accord with prior reports (Corbett 1989; Herberg and Rose 1989; Carlezon and Wise 1993; Carlezon and Wise 1996; Olds 1996; Cabeza de Vaca and Carr 1998; Sukhotina et al. 1999; De Vry et al. 2001; Sundstrom et al. 2002;

Kenny et al. 2003). This pattern of data indicates that blockade of NMDA receptors by MK-801 facilitates BSR without altering response performance.

One study failed to demonstrate a reinforcing effect of systemic MK-801, possibly due to the method used to measure reinforcement. Ranaldi et al. (1997) reported that MK-801 (0.05 mg kg^{-1} IP 30 min prior) did not change MFB BSR rate-frequency thresholds or maximum response rates, when threshold was defined as the lowest frequency that sustained responding.

In addition, the reward-increasing effect of MK-801 in the present study may extend to other noncompetitive NMDA receptor antagonists, such as memantine (see Tzschentke and Schmidt 1999).

7-OH-DPAT (0.03 mg kg^{-1} SC 15 min prior) increased BSR rate-frequency thresholds, indicating decreased reward

In the current study, 7-OH-DPAT (0.03 mg kg^{-1} 15 min prior) significantly increased rate-frequency thresholds, indicating decreased reward. Similar effects were reported by Kling-Petersen et al. (1995b). In that study, R-(+)-7-OH-DPAT induced biphasic effects on electrical self-stimulation of the MFB; doses between 0.0005-0.03 mg kg^{-1} increased rate-intensity thresholds while higher doses resulted in opposite effects. Gilbert et al. (1995) reported inhibition of response rates by 7-OH-DPAT (0.03-1.0 mg kg^{-1}); however response rates alone are difficult to interpret due to nonspecific performance effects. Depoortere et al. (1996) showed that 7-OH-DPAT (0.1 mg kg^{-1}) shifted rate-frequency curves to the right, indicating decreased reward. In a subsequent study, Depoortere et al. (1999) reported that 7-OH-DPAT (0.01-3.0 mg kg^{-1}) induced biphasic effects in rats responding for VTA BSR under a progressive-ratio schedule of reinforcement; a low dose (0.03 mg kg^{-1}) significantly decreased the breaking point, while higher doses (1.0 and 3.0 mg kg^{-1}) increased the breaking point. Baldo et al. (1999a) showed that 7-OH-DPAT (0.01-1.0 mg kg^{-1}) produced inconsistent rate-intensity threshold elevations of rats responding for electrical stimulation of the MFB-LH, while response latencies were significantly increased by the higher dose (1.0 mg kg^{-1}). Lastly, one study reported that 7-OH-DPAT (0.0025-0.074 mg kg^{-1}) did not significantly affect rate-frequency thresholds or maximal rates of responding (Hatcher and Hagan 1998). Given that this agonist has lower affinity for the D_2 DA receptor subtype relative to D_3 DA receptors (Lévesque et al. 1992), and prior studies have attributed inhibitory effects of low doses of 7-OH-DPAT to stimulation of inhibitory postsynaptic D_3 DA receptors (Waters et al. 1993; Svensson et al. 1994c), the BSR rate-frequency threshold elevating effect of 7-OH-DPAT seen in the present study may be related to stimulation of "behaviourally inhibitory" D_3 DA receptors located postsynaptically in the NAS.

Apomorphine (0.05 mg kg^{-1} SC 10 min prior) increased BSR rate-frequency thresholds, indicating decreased reward

In the present study, the effects of apomorphine (0.05 mg kg^{-1}) are consistent with prior reports that similar doses (0.01-0.1 mg kg^{-1}) increased rate-frequency thresholds of LH BSR (Fouriezos and Francis 1992). In addition, using a rate-independent discrete trial procedure, apomorphine (0.05 mg kg^{-1}) has been shown to raise MFB-LH BSR current intensity thresholds (Knapp and Kornetsky 1996). In general, the attenuation of the reinforcing effects of BSR by low doses of apomorphine may be attributed to activation of presynaptic D_2-like DA autoreceptors (Fouriezos and Francis 1992).

Effects of systemic administration of MK-801 and 7-OH-DPAT on BSR rate-frequency thresholds were additive

In the present study, there was no interaction between the effects of MK-801 and 7-OH-DPAT. Concurrent NMDA receptor blockade by MK-801 and stimulation of $D_{2/3}$ DA receptors by 7-OH-DPAT resulted in additive effects. These additive effects contrast with prior reports that demonstrated synergistic effects of systemically administered Glu receptor antagonists and cocaine. As described in Chapter 2, Ranaldi et al. (1997) reported that MK-801 (0.05 mg kg^{-1}) or cocaine (4 mg kg^{-1}) alone did not affect rate-frequency thresholds of LH BSR. When combined, these doses of MK-801 and cocaine reduced rate-frequency thresholds (i.e., increased reward). In addition, Tzschentke and Schmidt (2000) investigated the effects of another noncompetitive NMDA receptor antagonist, memantine, on cocaine-induced potentiation of MFB BSR. In that study, cocaine (10 mg kg^{-1}) decreased threshold frequency, and this effect was further enhanced by memantine (10 mg kg^{-1}), which alone was ineffective. Both Ranaldi et al. (1997) and Tzschentke and Schmidt (2000) concluded that NMDA receptor blockade may add to the stimulation of DA release induced by cocaine, or could reduce the activity of medium spiny neurons in the NAS to act in synergy with the DA-related effects of cocaine. The results of the present experiment with MK-801 and 7-OH-DPAT may suggest that differences in the DA receptor selectivity of cocaine vs. 7-OH-DPAT may contribute to the differential interactions of these ligands with Glu receptor antagonists. In addition, these results suggest that the reward-enhancing action of MK-801 in BSR is independent of stimulation of postsynaptic D_3 DA receptors.

Effects of systemic administration of MK-801 and apomorphine on BSR rate-frequency thresholds were additive

To the best of our knowledge, there are currently no reports of interactions between low dose apomorphine and glutamatergic compounds in BSR. Our finding that MK-801 and apomorphine effects do not interact is additional evidence for the possible independence of D_2-like DA receptors and NMDA receptors in the context of BSR. This finding is reinforced by an additional experiment from our laboratory (unpublished results) – using a similar design, we have observed no interaction between MK-801 and a higher dose of apomorphine (0.1 mg kg^{-1}) on VTA BSR rate-frequency thresholds. These results suggest that the reward-enhancing action of MK-801 in BSR is also independent of presynaptic D_2-like DA autoreceptors.

Conclusion

This study investigated the interaction between NMDA receptor blockade by MK-801 and DA receptor stimulation by 7-OH-DPAT or apomorphine using BSR as a measure of primary reinforcement. Administration of the selective $D_{2/3}$ DA agonist 7-OH-DPAT or low dose apomorphine increased BSR rate-frequency thresholds (i.e., decreased reward) and this effect counteracts facilitation of BSR by the NMDA receptor antagonist MK-801. Although these findings are limited to effects of D_2-like DA agonists, the present study is the first to demonstrate an independence of DA and Glu systems in the context of electrical brain self-stimulation. Specifically, the facilitation of VTA BSR by MK-801 is independent of both pre- and postsynaptic $DA_{2/3}$ receptor stimulation. The next chapter will assess the role of the NAS shell in DA-Glu interactions in this context by exploring the effects of Glu receptor blockade and $D_{2/3}$ DA receptor stimulation in the NAS shell on VTA BSR.

Chapter 5. Interaction between the $D_{2/3}$ DA receptor agonist 7-OH-DPAT and Glu receptor antagonists in the context of brain stimulation reward: NMDA vs. AMPA/kainate

(Parts of this chapter have been published in *Behavioural Brain Research* or *Psychopharmacology* – see Choi et al. 2005; Clements & Greenshaw 2005b)

Introduction

The experiments described in the previous chapter indicated that the effects of the NMDA receptor antagonist MK-801 and DA agonists 7-OH-DPAT and apomorphine on BSR were additive. The present chapter explored further the effects of Glu receptor antagonists and the DA agonist 7-OH-DPAT following direct administration of these ligands into the NAS.

As described previously, studies using DA receptor-related compounds to alter BSR have contributed significantly to our understanding of the role of DA in disorders of motivation. Although there is some evidence that contrasts with a simple direct role of DA in mediating processes underlying motivation and reward (Garris et al. 1999), BSR generated by VTA stimulation increases DA release in the NAS; in addition, activation of D_2-like DA receptors in the NAS may play a role in mediating the rewarding and/or motivating effects of VTA stimulation (Phillips et al. 1989; Fiorino et al. 1993). The NAS is of primary interest in the context of BSR, as DA-containing cell bodies of the VTA project to the NAS, as do Glu projections from the PFC (Sesack and Pickel 1992; Pennartz et al. 1994). The NAS, in addition to the VTA, islands of Calleja, and olfactory tubercle, contains a relatively high density of D_3 DA receptors (Lévesque et al. 1992; Diaz et al. 2000).

As described in Chapter 4, studies using systemic administration of the $D_{2/3}$ DA receptor agonist 7-OH-DPAT have failed to reveal consistent effects on BSR. However, since 7-OH-DPAT is the most selective agonist currently available to stimulate D_3 DA receptors, the effects of this compound on VTA BSR following intra-NAS shell administration have been investigated in the present study.

Despite the established role of Glu in the NAS in reward and motivation, there remain few reports describing the behavioural effects of Glu receptor-related compounds in the NAS on BSR. This is surprising in view of the importance of these receptors in regulating neuronal activity and the recognized usefulness of the BSR paradigm (Wise 1996). Carlezon and Wise (1996) demonstrated reward-enhancing effects of NMDA receptor antagonists in the NAS using MFB BSR, and subsequently Panagis and Kastellakis (2002) reported that intra-VTA administration of AMPA did not affect BSR of the ventral pallidum; however, there is currently only one report describing the effects of AMPA/kainate receptor antagonists on VTA BSR (Choi 2000). In that study, the AMPA/kainate receptor antagonist CNQX potentiated the reward-decreasing effect of intra-NAS shell 7-OH-DPAT. Further studies using AMPA receptor-related ligands are needed, as drugs of abuse have been shown to increase expression of GluR1 (an AMPA receptor subunit) in the VTA (Fitzgerald et al. 1996; Churchill et al. 1999), and BSR of the MFB decreases expression of this subunit in the VTA (Carlezon et al. 2001).

The purposes of the current investigation were to use BSR to assess: 1) effects of intra-NAS shell NMDA receptor antagonist MK-801 and AMPA/kainate receptor antagonist NBQX; 2) effects of intra-NAS shell 7-OH-DPAT; and 3) to characterize the interaction between NMDA receptors (or AMPA/kainate receptors) and $D_{2/3}$ DA receptors in the NAS shell.

Hypotheses

The hypotheses for experiments described in this chapter were as follows:

1. Intra-NAS shell administration of MK-801 would decrease BSR rate-frequency thresholds.
2. Intra-NAS shell administration of NBQX would not alter BSR rate-frequency thresholds.
3. Intra-NAS shell administration of 7-OH-DPAT would not increase BSR rate-frequency thresholds.
4. In the NAS shell, MK-801 or NBQX combined with 7-OH-DPAT would increase BSR rate-frequency thresholds.

Materials and methods

Animals

Male Sprague-Dawley rats (200-250 g; Health Sciences Laboratory Animal Services, University of Alberta) were housed individually in a temperature- (21±1°C) and humidity-controlled environment with a 12-hour light/dark cycle (lights on 0700-1900 hours). Food (LabDiet 5001 Rodent Diet, PMI Nutrition International Inc., Brentwood, MO, USA) and water were freely available. Separate groups of animals were used for each experiment, and testing always occurred between 0900-1700 hours. The care and use of animals was approved by the Health Sciences Animal Policy and Welfare Committee and the University Animal Policy and Welfare Committee at the University of Alberta, and complied with the "Guide to the Care and Use of Experimental Animals" (Volume 1, 2nd Edition, 1993; Volume 2, 1984) published by the Canadian Council on Animal Care (CCAC).

Drugs

(+)-MK-801 maleate [dizocilpine; (5S, 10R)-(+)-5-methyl-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5,10-imine maleate], 7-OH-DPAT ((±)-7-hydroxy-2-dipropylaminotetralin) hydrobromide, and NBQX (2,3-dioxo-6-nitro-1,2,3,4-tetrahydrobenzo[f]quinoxaline-7-sulfonamide disodium were obtained from Sigma RBI (Oakville, ON, CA). Compounds were dissolved in isotonic saline (Fisher Scientific Ltd., Nepean, ON, CA) and drug solutions were made daily (pH

5.0-7.0) according to the protocol in Appendix A. Artificial cerebrospinal fluid (CSF) was freshly prepared according to Lewis and Elliott (1950) (see Appendix B). Doses are expressed as free bases. The doses of NBQX and 7-OH-DPAT used in these experiments was based upon prior work in this laboratory (Choi 2000) while the dose of intra-NAS shell MK-801 was selected from the work of Carlezon and Wise (1996).

Stereotaxic surgery

A standard operating procedure for central implantation is provided in Appendix C. Briefly, under aseptic conditions, animals weighing between 300-350 g were anaesthetized using inhalation Halothane (2-bromo-2-chloro-1,1,1-trifluoroethane; Halocarbon Laboratories, River Edge, NJ, USA) and placed in a Kopf stereotaxic instrument (Kopf Instruments, Tujunga, CA, USA). All animals were implanted bilaterally with stainless steel 22 gauge guide cannulae (C313G, Plastics One Inc., Roanoke, VA, USA) directed at the NAS shell subregion, in addition to a unilateral monopolar stimulating electrode (E363/2, Plastics One Inc., Roanoke, VA, USA; diameter 200 μm) directed at the VTA. Stereotaxic coordinates were interpolated from the target site (defined by Paxinos and Watson 1986) using angular placements to avoid damage to blood sinuses and cerebral ventricles (Greenshaw 1997b). These coordinates are relative to inter-aural zero with the incisor bar set 2.4 mm below the inter-aural line: NAS shell AP +10.1 mm, LM ±0.2 mm, DV +3.7 mm, 16° lateral from sagittal plane; VTA AP +3.2

mm, LM +0.6 mm, DV +2.3 mm, 20° lateral and 20° anterior from sagittal and coronal planes respectively. Tips of guide cannulae were placed 1.0 mm above the target site. In addition, an indifferent electrode (E363/0, Plastics One Inc., Roanoke, VA, USA) with silver wire was fixed to the frontal bone. The electrodes and cannulae were fixed to the skull using stainless steel screws (Lomat Watch Co., Montréal, QC, CA) and dental acrylic (Caulk/Dentsply, Milford, DE, USA). Dummy cannulae (C313DC, Plastics One Inc., Roanoke, VA, USA) were left in place between microinjection procedures to prevent occlusion. Training began at least one week after surgery.

Apparatus

Experiments were carried out in ventilated operant test chambers (24 cm L x 30 cm W x 29 cm H) each equipped with a sound-attenuating outer chamber (Coulbourn Instruments Ltd., Lehigh Valley, PA, USA). Gold track slip rings allowed free movement of the animal and were connected to constant current programmable stimulators (I. Halvorsen Systems Design, Phoenix, AZ, USA). Stimulation parameters and recording of behavioural responses were under computer control. Between pulses, the stimulating and ground electrodes were connected through a 10 kΩ resistor to cancel any effects of electrode polarization. Throughout the experiment, each animal was always exposed to the same apparatus that was cleaned thoroughly between animals using diluted ammonia-based window cleaner (1:6 in water) (No Name Glass Cleaner with Ammonia, Loblaw Companies Ltd., Toronto, ON).

Training and baseline procedures

Following recovery, each animal was placed in the apparatus and trained to lever-press for electrical stimulation of the VTA (cathodal pulse width 200 ms; frequency 100 Hz; train length 1 s). Initially, the animal's exploratory behaviour was shaped to determine a current intensity (80-400 μA) that maintained lever-pressing. Once established, daily training sessions on continuous reinforcement schedules allowed each animal to associate lever-pressing with brain stimulation. Once responding stabilized, animals underwent daily sessions designed to determine the stimulating current that produced half-maximal responding, while stimulation frequency was held constant at 100 Hz. This was determined using rate-intensity analysis that consisted of stepwise changes in current every 2 minutes while all other parameters remained constant. This regimen continued until the half-maximal current varied less than 10% for three consecutive days. For each animal, this current was used for all subsequent daily sessions. Animals then underwent daily sessions designed to determine the stimulation frequency (0-160 Hz) that produced half-maximal responding (rate-frequency threshold or M_{50}). One rate-frequency function was generated per day, using rate-frequency analysis consisting of stepwise changes in frequency while other parameters, including current, remained constant. Responding was initiated at 160 Hz and decreased stepwise each minute by 0.1 log Hz until 0 Hz (or until the animal stopped responding for the duration of a single frequency). Stimulation frequency was then reversed to increase stepwise each minute by 0.1 log Hz up to 160 Hz. For each animal, the descending and ascending segments were collapsed to generate a single rate-frequency function; linear regression was used to determine M_{50}. This regimen was continued until the M_{50} varied less than 10% across three consecutive days. The M_{50}, maximum response rate (RMAX), and total responses (TRES) were recorded during all subsequent daily sessions. At the beginning of each time bin for rate-intensity and rate-frequency analysis, each animal received three trains of stimulation (primes) that served as a discriminative stimulus to indicate that stimulation was available. This method of measuring frequency thresholds provides an objective measure of reward sensitivity that is easily distinguished from nonspecific changes in behaviour, while the maximum response rate is a measure of response performance (Gallistel and Karras 1984; Greenshaw and Wishart 1987).

Microinjection procedure

For each microinjection, dummy cannulae were carefully removed and 28 gauge injection cannulae (C313I, Plastics One Inc., Roanoke, VA, USA) were slowly lowered into the guide cannulae. The tip of each injection cannula extended 1.0 mm beyond the tip of the guide cannula. Bilateral microinjections (0.5 μL per side at a rate of 0.2 μL per min) were delivered over 2.5 minutes using

0.03 mL per meter Accu-rated pump tubes (Fisher Scientific Ltd., Nepean, ON, CA) and 10 μL glass microsyringes (Hewlett-Packard, Mississauga, ON, CA) attached to a Bee Hive Controller (Bioanalytical Systems Inc., West Lafayette, IN, USA). The injection cannulae remained in place for one minute following infusion to allow for drug absorption and diffusion. Microinjection was immediately followed by behavioural procedures.

Experimental designs

Two separate groups of animals received a randomized series of microinjections according to a repeated measures design with 3 days of baseline testing between drug treatments. Animals received a counterbalanced sequence of: CSF, MK-801 (0.66 μg), 7-OH-DPAT (5.0 μg), and MK-801 + 7-OH-DPAT (experiment 6, n=10) or CSF, NBQX (0.5 μg), 7-OH-DPAT (5.0 μg), and NBQX + 7-OH-DPAT (experiment 7, n=15).

Histology

Animals were deeply anaesthetized with sodium pentobarbital (Bimeda-MTC Animal Health Inc., Cambridge, ON, CA; Somnotol or Euthanyl) and perfused intracardially with 50 mL ice-cold isotonic saline (Fisher Scientific Ltd., Nepean, ON, CA) followed by 50 mL 10% w/v buffered formalin phosphate (Fisher Scientific Ltd., Nepean, ON, CA). Following decapitation, brains were removed and stored at room temperature in 10% w/v buffered formalin phosphate for 4-6 hours, then stored in 30% sucrose/10 mM PBS buffer for at least 24 hours. Brains were then flash-frozen by immersion in isopentane (2-methylbutane; Sigma-Aldrich Co., Oakville, ON, CA) chilled on solid carbon dioxide (Praxair Canada Inc., Edmonton, AB, CA). Brains were briefly dried and stored at –80°C until sectioning. Cannula and electrode placements were verified by inspection of 40 µm coronal brain sections. This histology protocol is described further in Appendix D. Only animals with correct cannula and electrode placements were included in data analyses. Representative photomicrographs of intra-NAS shell microinjection sites and intra-VTA electrode tips are shown in Appendices E and F respectively.

Statistical analyses

Data are presented as % of baseline response (M_{50}, RMAX, or TRES) defined as the mean of data collected during the drug-free days prior to each treatment (as noted above), and all analyses were completed using these % baseline data. In experiment 6, effects of MK-801 and 7-OH-DPAT on each measure were assessed by 2 x 2 repeated measures ANOVA (MK-801 x 7-OH-DPAT). Similarly, in experiment 7, effects of NBQX and 7-OH-DPAT on each measure were determined using a 2 x 2 repeated measures ANOVA (NBQX x 7-OH-DPAT). Simple effects were explored following significant NBQX x 7-OH-DPAT interaction using matched-sample t-tests with Bonferroni adjustments.

Statistical analyses ($\alpha=0.05$) were completed using statistical software (SPSS Inc., Chicago, IL, USA).

For each repeated measures experiment, Greenhouse-Geisser adjusted degrees of freedom have been used; because of this, degrees of freedom values may appear as non-integers.

Rate-frequency regression curves

For illustrative purposes, group-averaged rate-frequency curves were derived by converting response rate per frequency to a percentage of maximum response rate for each animal, and then averaging the percentages for each frequency by treatment group. In accordance with prior work from this laboratory (Choi 2000), non-linear regression curves were generated by Prism software (GraphPad Software, Inc., San Diego, CA, USA) using the sigmoidal dose-response equation with a variable slope:

$$y = \mathrm{min} + \frac{\mathrm{max} - \mathrm{min}}{1 + 10^{(\mathrm{logEC50} - x)\mathrm{Hillslope}}}$$

It is noteworthy that, as described above, M_{50} was calculated for individual animals each day using linear regression; illustrative group-averaged rate-frequency curves derived from non-linear regression are used for a convenient visual summary of performance. While regression results are usually concordant, discrepancies may arise due to differences in regression equation parameters.

Results

Experiment 6: Effects of intra-NAS shell MK-801 and 7-OH-DPAT on VTA BSR

Histological locations of NAS shell microinjection sites and VTA electrode placements are shown in Figures 5.1 and 5.2 respectively.

The general pattern of results indicates that there was no interaction between the effects of MK-801 and 7-OH-DPAT on BSR on any measure; effects of MK-801 and 7-OH-DPAT were additive.

Two-way repeated measures ANOVA revealed that there were significant main effects of MK-801 (F(1,9)=5.411, P=0.045) and 7-OH-DPAT (F(1,9)=12.891, P=0.006) on M_{50}, but there was no significant MK-801 x 7-OH-DPAT interaction (F(1,9)=0.164, P=0.695) on this measure (Figure 5.3). There was a significant main effect of 7-OH-DPAT on RMAX (F(1,9)=28.745, P<0.001), but not of MK-801 (F(1,9)=3.664, P=0.088) nor was there significant MK-801 x 7-OH-DPAT interaction (F(1,9)=0.080, P=0.783) on this measure (Figure 5.4). There were significant main effects of MK-801 (F(1,9)=6.902, P=0.027) and 7-OH-DPAT (F(1,9)=26.440, P=0.001) on TRES, but there was no significant MK-801 x 7-OH-DPAT interaction (F(1,9)=0.702, P=0.424) on this measure (Figure 5.5).

As shown in Figure 5.6, intra-NAS shell MK-801 shifted the rate-frequency curve to the left, indicating increased reward, while 7-OH-DPAT had opposite effects. Co-administration of MK-801 and 7-OH-DPAT into the NAS shell also shifted the rate-frequency curve to the right, indicating increased BSR rate-frequency thresholds, or decreased reward.

Figure 5.1. Illustration of histological verification of microinjection sites in the NAS shell subregion. Numbers represent distances in the coronal plane from interaural zero according to the atlas of Paxinos and Watson (1986).

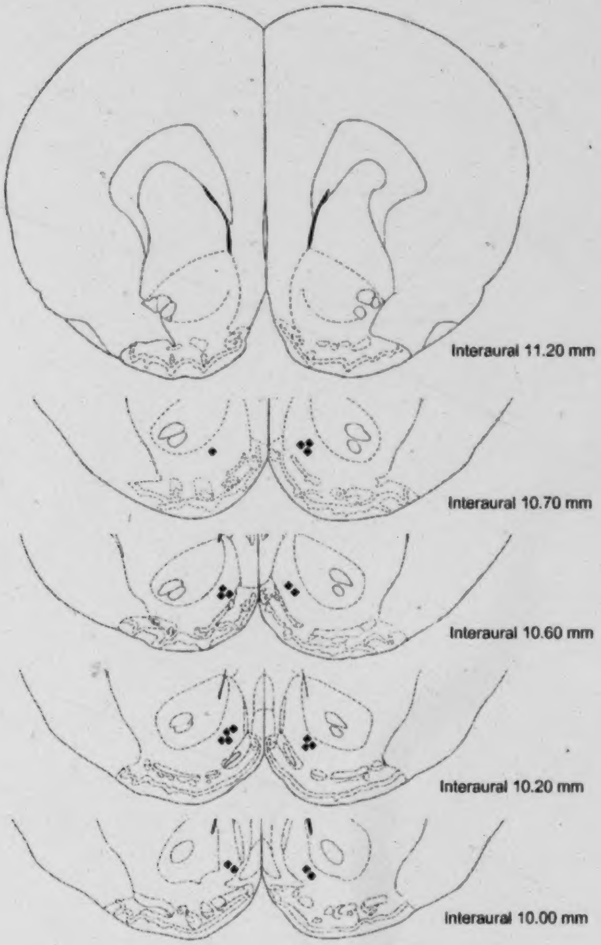

Interaural 11.20 mm
Interaural 10.70 mm
Interaural 10.60 mm
Interaural 10.20 mm
Interaural 10.00 mm

Figure 5.2. Illustration of histological verification of electrode sites in the VTA. Numbers represent distances in the coronal plane from interaural zero according to the atlas of Paxinos and Watson (1986).

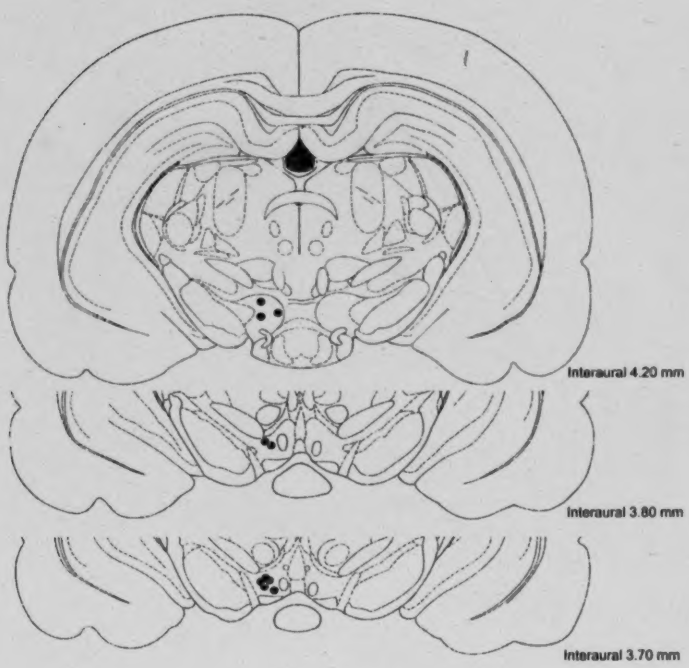

Interaural 4.20 mm
Interaural 3.80 mm
Interaural 3.70 mm

Figure 5.3. Effects of intra-NAS shell MK-801 (0.66 µg) and 7-OH-DPAT (5.0 µg) on BSR rate-frequency thresholds (M_{50}). Data are presented as mean ± SEM (n=10). Significant main effect (*) at P<0.05. M+D refers to the combination of MK-801 and 7-OH-DPAT.

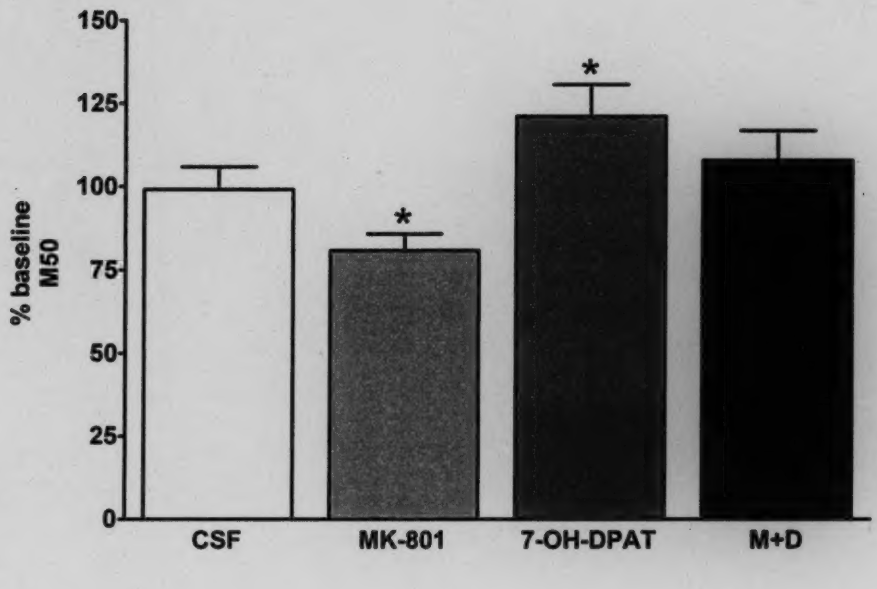


Figure 5.4. Effects of intra-NAS MK-801 (0.66 μg) and 7-OH-DPAT (5.0 μg) on BSR maximum response (RMAX). Data are presented as mean ± SEM (n=10). Significant main effect (*) at P<0.05. M+D refers to the combination of MK-801 and 7-OH-DPAT.

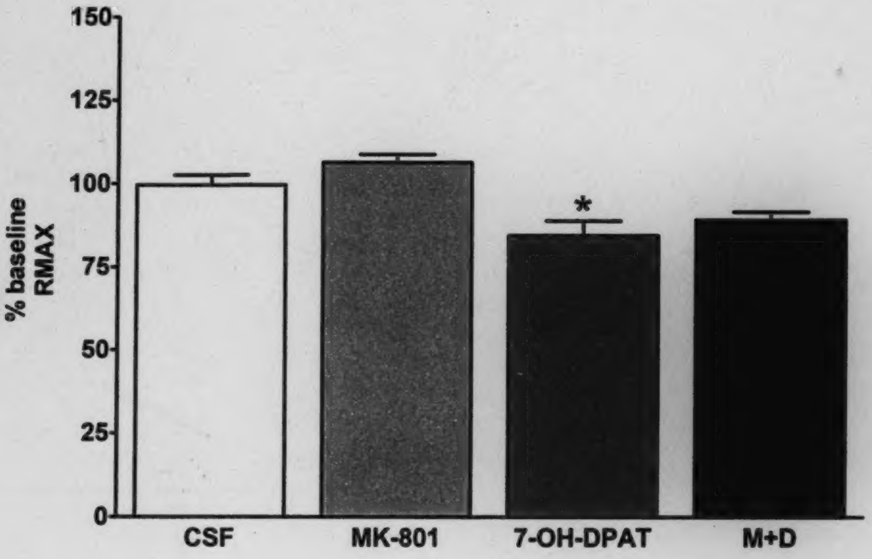


Figure 5.5. Effects of intra-NAS MK-801 (0.66 µg) and 7-OH-DPAT (5.0 µg) on BSR total responses (TRES). Data are presented as mean ± SEM (n=10). Significant main effect (*) at P<0.05. M+D refers to the combination of MK-801 and 7-OH-DPAT.

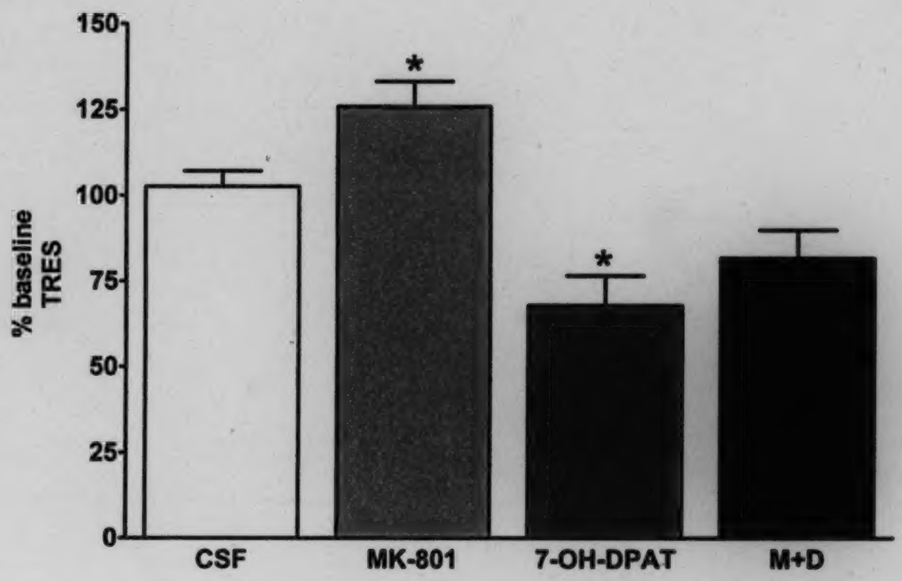
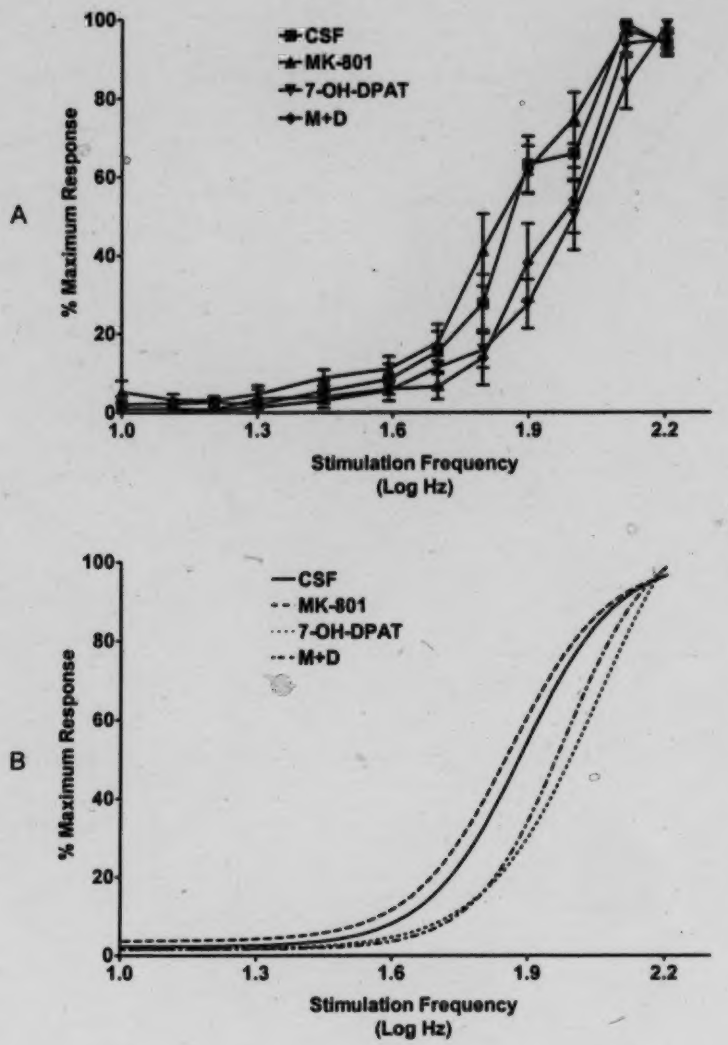


Figure 5.6. Rate-frequency data (A) and non-linear regression curves (B) for MK-801 (0.66 μg) and 7-OH-DPAT (5.0 μg). Data are presented as mean ± SEM (n=10). M+D refers to the combination of MK-801 and 7-OH-DPAT.



Experiment 7: Effects of intra-NAS shell NBQX and 7-OH-DPAT on VTA BSR

Histological locations of NAS shell microinjection sites and VTA electrode placements are shown in Figures 5.7 and 5.8 respectively.

The general pattern of results indicates that there was a significant interaction between effects of NBQX and 7-OH-DPAT.

Two-way repeated measures ANOVA failed to reveal significant main effects of NBQX (F(1,14)=0.430, P=0.523) or 7-OH-DPAT (F(1,14)=4.226, P=0.059) on M_{50}, but indicated a significant NBQX x 7-OH-DPAT interaction (F(1,14)=7.686, P=0.015) on this measure (Figure 5.9). Tests of simple effects revealed that 7-OH-DPAT alone was ineffective (t(14)=0.301, P=0.768) but increased BSR rate-frequency thresholds in the presence of NBQX (t(14)=3.425, P=0.004). Similarly, NBQX was not effective alone (t(14)=1.336, P=0.203) but increased BSR rate-frequency thresholds in the presence of 7-OH-DPAT (t(14)=2.537, P=0.024).

There was a significant main effect of 7-OH-DPAT on RMAX (F(1,14)=11.969, P=0.004), but there was no main effect of NBQX (F(1,14)=1.435, P=0.251) nor NBQX x 7-OH-DPAT interaction (F(1,14)=1.973, P=0.182) on this measure (Figure 5.10).

Two-way repeated measures ANOVA failed to reveal a significant main effect of NBQX (F(1,14)=1.894, P=0.190) on TRES, but there was a significant main effect of 7-OH-DPAT (F(1,14)=9.624, P=0.008) and significant NBQX x 7-OH-DPAT interaction (F(1,14)=6.447, P=0.024) on this measure (Figure 5.11).

Tests of simple effects revealed that 7-OH-DPAT alone was ineffective (t(14)=0.953, P=0.357) but decreased TRES in the presence of NBQX (t(14)=4.363, P=0.001). Similarly, NBQX was not effective alone (t(14)=1.077, P=0.300) but decreased TRES in the presence of 7-OH-DPAT (t(14)=2.942, P=0.011).

As shown in Figure 5.12, co-administration of NBQX and 7-OH-DPAT into the NAS shell shifted the rate-frequency curve to the right, indicating increased BSR rate-frequency thresholds, or decreased reward. There is a discrepancy between this figure and the histogram presentation of M_{50} (Figure 5.9) that is related to differences in regression analysis (see *Methods*). While drug effects were determined by assessment of changes in M_{50} for individual animals (Figure 5.9), group-averaged rate-frequency curves (Figure 5.12) are included for completeness.

Figure 5.7. Illustration of histological verification of microinjection sites in the NAS shell subregion. Numbers represent distances in the coronal plane from interaural zero according to the atlas of Paxinos and Watson (1986).

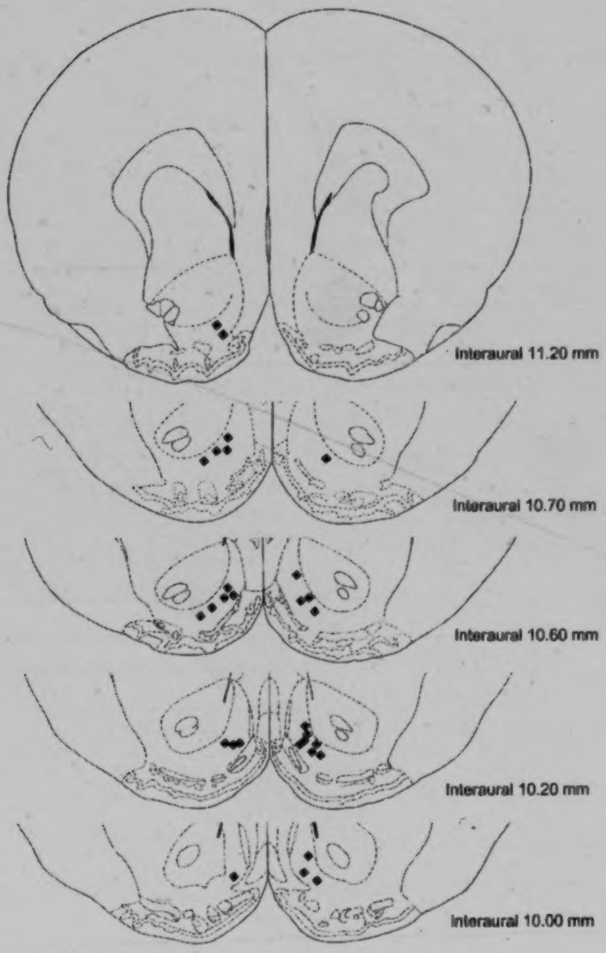

Interaural 11.20 mm
Interaural 10.70 mm
Interaural 10.60 mm
Interaural 10.20 mm
Interaural 10.00 mm

Figure 5.8. Illustration of histological verification of electrode sites in the VTA. Numbers represent distances in the coronal plane from interaural zero according to the atlas of Paxinos and Watson (1986).

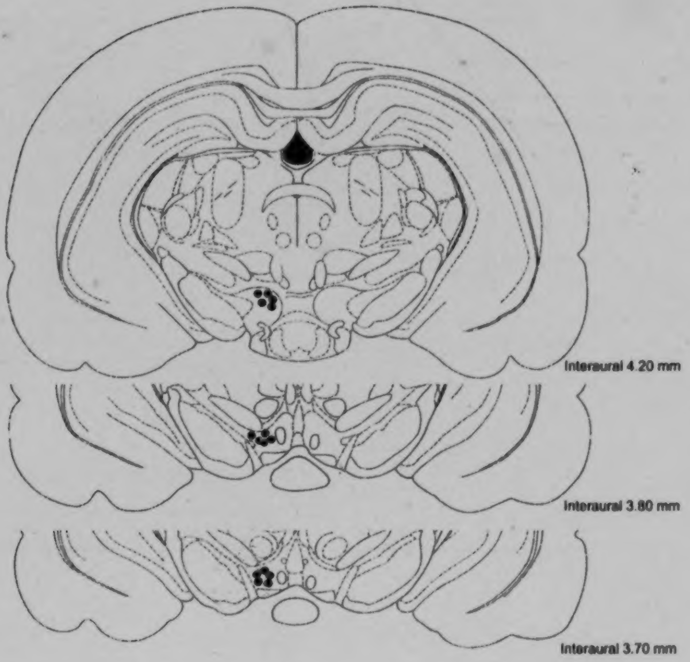

Interaural 4.20 mm
Interaural 3.80 mm
Interaural 3.70 mm

Figure 5.9. Effects of intra-NAS shell NBQX (0.5 μg) and 7-OH-DPAT (5.0 μg) on BSR rate-frequency thresholds (M_{50}). Data are presented as mean ± SEM (n=15). Significant interaction (★) at $P<0.05$. Simple effects of NBQX (a) and 7-OH-DPAT (b). N+D refers to the combination of NBQX and 7-OH-DPAT.

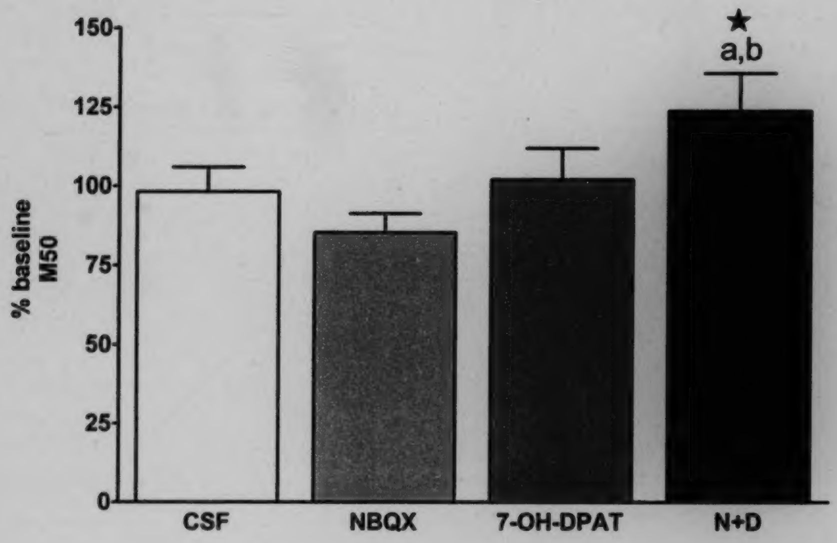


Figure 5.10. Effects of intra-NAS NBQX (0.5 µg) and 7-OH-DPAT (5.0 µg) on BSR maximum response (RMAX). Data are presented as mean ± SEM (n=15). Significant main effect (*) at P<0.05. N+D refers to the combination of NBQX and 7-OH-DPAT.

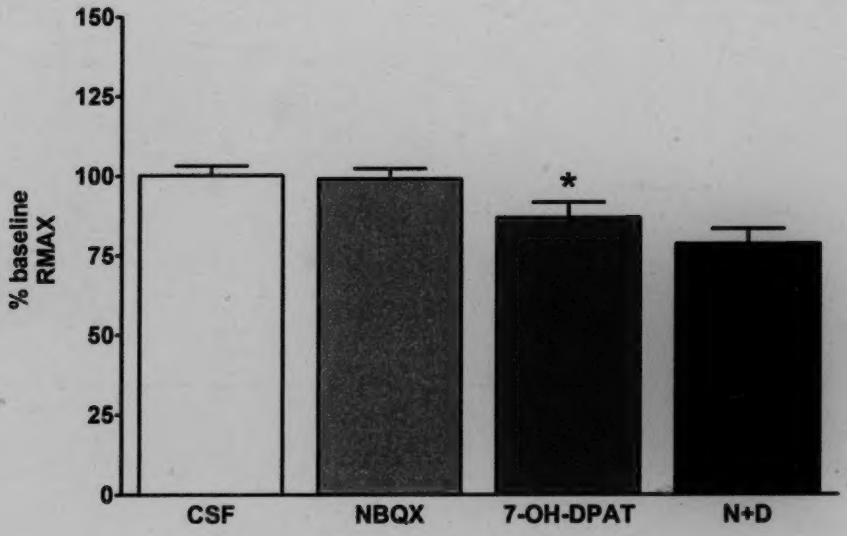


Figure 5.11. Effects of intra-NAS NBQX (0.5 μg) and 7-OH-DPAT (5.0 μg) on BSR total responses (TRES). Data are presented as mean ± SEM (n=15). Significant interaction (★) at P<0.05. Simple effects of NBQX (a) and 7-OH-DPAT (b). N+D refers to the combination of NBQX and 7-OH-DPAT.

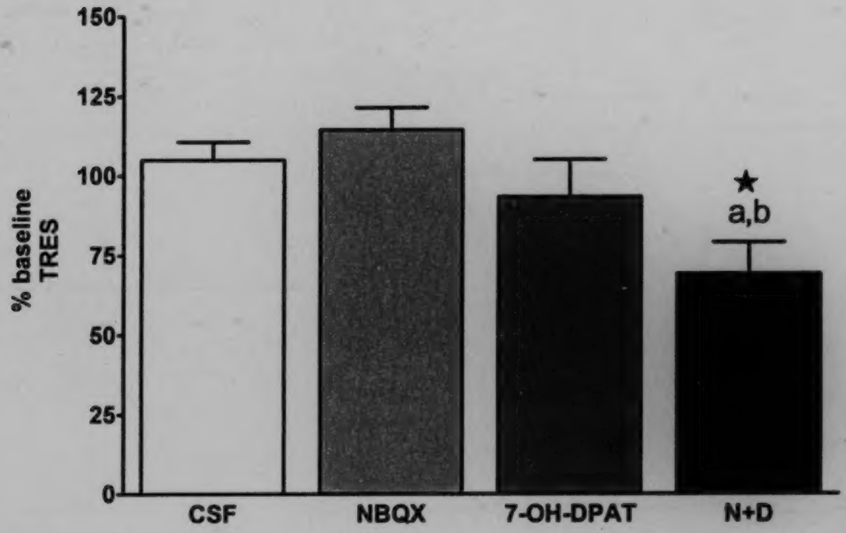
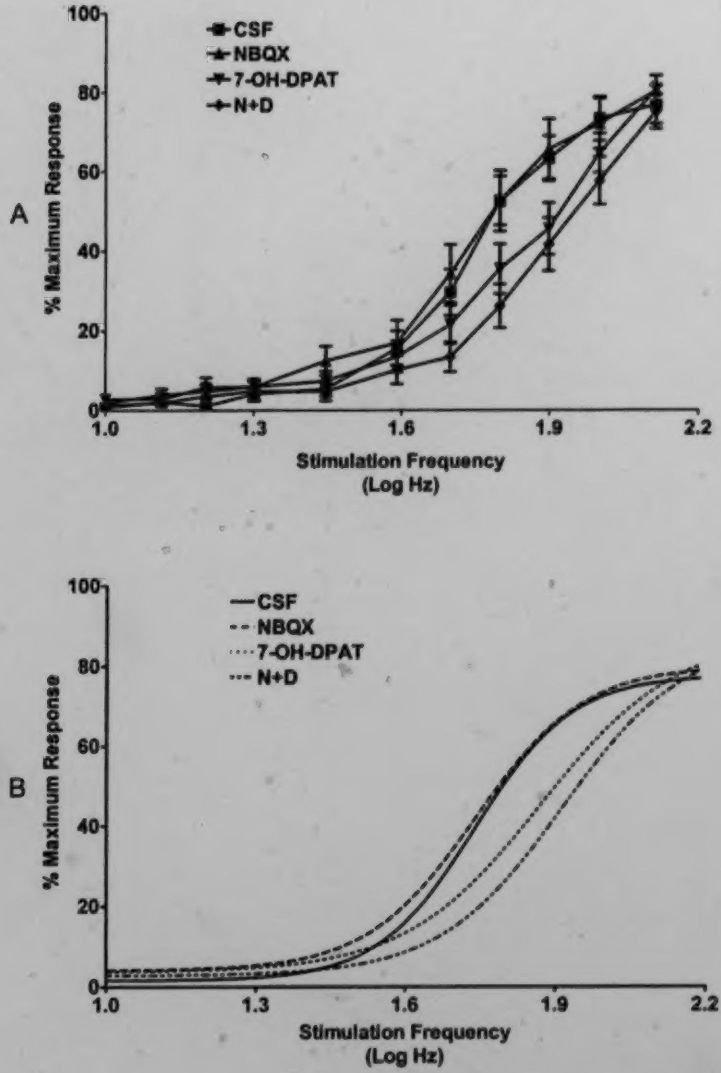


Figure 5.12. Rate-frequency data (A) and non-linear regression curves (B) for NBQX (0.5 μg) and 7-OH-DPAT (5.0 μg). Data are presented as mean ± SEM (n=15). N+D refers to the combination of NBQX and 7-OH-DPAT.



Discussion

The main findings of this chapter are listed below. Each finding will be briefly discussed in turn in this chapter. Discussion that relates the separate studies to each other is reserved for Chapter 7 (General Discussion).

- Intra-NAS shell MK-801 (0.66 μg) decreased BSR rate-frequency thresholds without altering response performance (Experiment 6).
- Intra-NAS shell NBQX (0.5 μg) did not affect BSR rate-frequency thresholds and did not alter response performance (Experiment 7).
- Intra-NAS shell 7-OH-DPAT (5.0 μg) had inconsistent effects on BSR rate-frequency thresholds, but decreased response performance in both studies (Experiments 6 and 7).
- Effects of intra-NAS shell MK-801 (0.66 μg) and 7-OH-DPAT (5.0 μg) on BSR rate-frequency thresholds did not interact; effects were opposite and additive (Experiment 6).
- Intra-NAS shell NBQX (0.5 μg) and 7-OH-DPAT (5.0 μg) resulted in a significant interaction on BSR rate-frequency thresholds. When combined, these compounds increased BSR rate-frequency thresholds, indicating decreased reward (Experiment 7).

Intra-NAS shell MK-801 (0.66 μg) decreased BSR rate-frequency thresholds without altering response performance

In the present study, MK-801 decreased rate-frequency thresholds for VTA BSR, in accord with prior reports of systemic drug effects (Corbett 1989; Herberg and Rose 1989; Carlezon and Wise 1993; Carlezon and Wise 1996; Olds 1996; Cabeza de Vaca and Carr 1998; Sukhotina et al. 1999; De Vry et al. 2001; Sundstrom et al. 2002; Kenny et al. 2003). This pattern of data indicates that blockade of NMDA receptors by MK-801 facilitates BSR without altering response performance. In addition, the present results extend the findings of the previous chapter to indicate that the NAS shell subregion is a sufficient site for this reward-increasing effect of MK-801. The rate-frequency threshold decreasing effect of MK-801 in the NAS was previously reported in one study that used hypothalamic BSR. In that study, intra-NAS shell administration of NMDA receptor antagonists (PCP, MK-801 or CPP) decreased rate-frequency thresholds (Carlezon and Wise 1996).

Intra-NAS shell NBQX (0.5 μg) did not affect BSR rate-frequency thresholds and did not alter response performance

In this study, administration of the AMPA/kainate receptor antagonist NBQX into the NAS shell did not affect BSR behaviour; no changes in BSR rate-frequency threshold or maximum response rates were observed. This is consistent with the lack of effect of this dose of NBQX in the NAS shell on spontaneous locomotor activity (Choi et al. 2000). In addition, the inability of NBQX to alter BSR rate-frequency thresholds agrees with recent evidence that intra-NAS shell administration of NBQX does not induce conditioned place preference or aversion (Biondo 2002). Most studies agree that AMPA/kainate receptor antagonists may be inactive in behavioural tests using experimentally naïve rats (Hauber and Andersen 1993; Kaddis et al. 1993; Boldry et al. 1993; Danysz et al. 1994; Bubser et al. 1995; Li et al. 1997; Mathé et al. 1998), although some studies have reported that another AMPA/kainate receptor antagonist, CNQX, decreases (Maj et al. 1995a) or increases locomotor activity following administration into the ventral striatum (Burns et al. 1994) or the NAS core subregion (David et al. 2004).

Intra-NAS shell 7-OH-DPAT (5.0 μg) had inconsistent effects on BSR rate-frequency thresholds, but decreased response performance in both studies

In this study, administration of the $D_{2/3}$ DA receptor agonist 7-OH-DPAT into the NAS did not alter rate-frequency thresholds of VTA BSR, although maximum response rates were reduced. That 7-OH-DPAT alone does not alter BSR rate-frequency thresholds despite its rate-decreasing effect is further evidence that our measure of reward is independent of changes in maximal response performance (Greenshaw and Wishart 1987). The lack of effect of intra-NAS shell 7-OH-DPAT in BSR is a replication of previous work from this laboratory (Choi 2000).

Two studies of systemic 7-OH-DPAT effects may support these findings. Baldo et al. (1999a) showed that 7-OH-DPAT (0.01-1.0 mg kg^{-1}) produced inconsistent rate-intensity threshold elevations of rats responding for electrical stimulation of the MFB-LH, while response latencies were significantly increased by a higher dose (1.0 mg kg^{-1}). Another study reported that 7-OH-DPAT (0.0025-0.074 mg kg^{-1}) did not significantly affect rate-frequency thresholds or maximal rates of responding (Hatcher and Hagan 1998). Given that 7-OH-DPAT has 15- to 20-fold greater selectivity for the low-affinity DA D_3 relative to D_2 DA receptors, and 5- to 12-fold greater selectivity for the high-affinity states of these receptors (Neve and Neve 1997), it is likely that the behavioural effects observed in this study are due to preferential stimulation of the former. This adds to the current literature that provides evidence for "behaviourally-inhibitory" D_3 DA receptors, that may be located postsynaptically (Waters et al. 1993; Svensson et al. 1994b,c; Mattingly et al. 1996; Depoortere et al. 1996; Zarandi 1998).

Although intra-NAS effects of 7-OH-DPAT have not previously been reported in the context of BSR (except Choi 2000), results using other $D_{2/3}$ DA receptor agonists are available. Singh et al. (1997), using a rate-frequency analysis, reported decreased VTA BSR rate-frequency thresholds following intra-NAS administration of the $D_{2/3}$ DA receptor agonist quinpirole. As quinpirole is a D_2-like DA agonist, it could be suggested that stimulation of D_2 rather than D_3 DA receptors underlies the changes in brain self-stimulation. In addition, the lack of an effect of 7-OH-DPAT alone in the NAS is reinforced by studies of intra-

NAS administration of the D_2-like DA receptor antagonist raclopride and D_3 DA receptor antagonist (+)-UH232. Raclopride has been shown to increase rate-frequency thresholds, whereas (+)-UH232 may not (Nakajima and Patterson 1997). Taken together, these studies suggest a role for direct stimulation or blockade of intra-NAS D_2 DA receptors, but not D_3 DA receptors, in brain stimulation reward.

Effects of intra-NAS shell MK-801 (0.66 μg) and 7-OH-DPAT (5.0 μg) on BSR rate-frequency thresholds are opposite and additive

In the present study, effects of intra-NAS shell MK-801 and 7-OH-DPAT did not interact. Concurrent intra-NAS shell NMDA receptor blockade by MK-801 and stimulation of $D_{2/3}$ DA receptors by 7-OH-DPAT resulted in additive effects. These additive effects concur with the systemic drug experiments in the previous chapter that demonstrated independence between effects of MK-801 and 7-OH-DPAT. In this study, the independence of MK-801 effects from DA receptor stimulation was also observed when these compounds were given directly into the NAS. Similar to the experiments described in Chapter 4, these additive effects contrast with a prior report that demonstrated rate-frequency threshold decreasing effects of a combination of the nonselective DA agonist cocaine and NMDA receptor blockade on lateral hypothalamic BSR (Ranaldi et al. 1997; Tzschentke and Schmidt 2000).

Intra-NAS shell NBQX (0.5 µg) and 7-OH-DPAT (5.0 µg) acted synergistically to increase BSR frequency thresholds, indicating decreased reward

In this experiment, there was an interaction between intra-NAS NBQX and 7-OH-DPAT; combined administration of these compounds decreased reward, without synergistically affecting maximum response rates. In other words, the reduction in maximum response rates caused by 7-OH-DPAT was not changed by NBQX. These results suggest that the interaction seen between effects of APMA/kainate receptor blockade and $D_{2/3}$ DA receptor stimulation on BSR rate-frequency thresholds is not due to nonspecific effects on response performance. In addition, the BSR rate-frequency regression curves reveal that, compared to control treatment, the rate-frequency group-averaged responses for intra-NAS shell experiments are equally maximal, suggesting that none of the treatments significantly inhibited the animals' maximal performance when given into the NAS shell.

These results confirm previous experiments in our laboratory that demonstrated reward-decreasing effects of combined intra-NAS shell 7-OH-DPAT and CNQX, and provide further evidence for the proposal that combined blockade of AMPA/kainate receptors and stimulation of $D_{2/3}$ DA receptors in the NAS shell subregion may decrease motivated behaviour (Choi 2000; Choi et al. 2000).

Conclusion

This study investigated the interaction between NMDA or AMPA/kainate receptor blockade and DA receptor stimulation in the NAS shell using VTA BSR. There was no interaction between effects of MK-801 + 7-OH-DPAT in the NAS shell subregion, supporting an independence of DA and Glu systems in BSR following systemic drug administration (see Chapter 4). In contrast, there was significant interaction between the effects of 7-OH-DPAT and AMPA/kainate receptor blockade, in that co-administration of 7-OH-DPAT and NBQX decreased BSR. These findings may suggest that there may be a demonstrable interaction between D_3 DA and AMPA/kainate receptors in the mesolimbic system in the context of primary reinforcement. The next chapter will extend our investigation of DA-Glu interactions by measuring drug effects on secondary (or conditioned) reinforcement using indices of place conditioning.

Chapter 6. Unbiased place conditioning and conditioned locomotor effects of $D_{2/3}$ DA receptor stimulation and NMDA receptor antagonism

(A version of this chapter has been accepted for publication in *Psychopharmacology* – see Biondo et al. 2005)

Introduction

Place conditioning is a classical conditioning phenomenon in which animals are given a drug in one neutral environment and saline in an alternate neutral environment. When later allowed to explore either environment, a preference for the drug-paired environment indicates conditioned reward. Conversely, an aversion to the drug-paired environment may indicate conditioned aversion. Place conditioning is a widely accepted procedure for exploring reward-related neural processes in vivo.

Compounds that enhance DA transmission in the NAS (e.g., amphetamine or cocaine) consistently induce conditioned place preferences (CPP, e.g., Spyraki et al. 1982; Biała and Langwiński 1996; Bardo et al. 1999). More selective DA agonists such as 7-OH-DPAT, that has >100, >1000, and >10,000-fold selectivity for D_3 over D_2, D_4 and D_1 DA receptors respectively (Lévesque et al. 1992), may also induce CPP (Mallet and Beninger 1994; Kling-Petersen et al. 1995b; Chaperon and Thiébot 1996). This effect is likely related to the localization of D_3

DA receptors to DA terminal areas of the limbic forebrain, including the NAS (Mengod et al. 1992; Sokoloff et al. 1992).

There is evidence to suggest that Glu receptor antagonists may affect CPP induced by DA receptor agonists (see Chapter 2). For example, MK-801 may reduce CPP induced by methamphetamine or cocaine (Cervo and Samanin 1995; Kim et al. 1996; Kim and Jang 1997) while NBQX may prevent the induction of CPP to amphetamine or 7-OH-DPAT (Mead and Stephens 1999; Biondo 2002). Such studies indicate the importance of DA-Glu interactions in this context.

Using relatively receptor-specific compounds, the present study examined the effects of NMDA receptor blockade (by MK-801) and $D_{2/3}$ DA receptor stimulation (by 7-OH-DPAT) alone and combined on place conditioning. As conditioned increases in activity may contribute to the development of CPP (Bozarth and Wise 1981; Vezina and Stewart 1987), conditioned locomotor responses to MK-801 and 7-OH-DPAT were also investigated.

Hypotheses

The hypotheses for experiments described in this chapter were as follows:

1. Systemic MK-801 alone would not induce place conditioning.
2. A high dose of systemic 7-OH-DPAT alone would induce CPP.
3. A low dose of systemic 7-OH-DPAT would induce CPA.
4. MK-801 would block place conditioning induced by 7-OH-DPAT.
5. Neither compound would induce conditioned locomotor effects.

Materials and methods

Animals

Male Sprague-Dawley rats (200-250 g; Health Sciences Laboratory Animal Services, University of Alberta) were housed individually in a temperature- (21±1°C) and humidity-controlled environment with a 12-hour light/dark cycle (lights on 0700-1900 hours). Food (LabDiet 5001 Rodent Diet, PMI Nutrition International Inc., Brentwood, MO, USA) and water were freely available. Separate groups of animals were used for each experiment, and testing always occurred between 0900-1700 hours. The care and use of animals was approved by the Health Sciences Animal Policy and Welfare Committee and the University Animal Policy and Welfare Committee at the University of Alberta, and complied with the "Guide to the Care and Use of Experimental Animals" (Volume 1, 2nd Edition, 1993; Volume 2, 1984) published by the Canadian Council on Animal Care (CCAC).

Drugs

(+)-MK-801 maleate [dizocilpine; (5S, 10R)-(+)-5-methyl-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5,10-imine maleate] and 7-OH-DPAT ((±)-7-hydroxy-2-dipropylaminotetralin) hydrobromide were obtained from Sigma RBI (Oakville, ON, CA). Compounds were dissolved in isotonic saline (Fisher Scientific Ltd., Nepean, ON, CA) and drug solutions were made daily (pH 5.0-7.0) according to the protocol in Appendix A. MK-801 (given IP, 15 minutes prior to testing) and 7-OH-DPAT (given SC, 15 minutes prior to testing) were injected in a volume of 1.0 mL kg^{-1}. Doses are expressed as free bases and, for 7-OH-DPAT, were determined from prior work in this laboratory (Biondo 2002).

Apparatus – Place conditioning

Plexiglas place conditioning boxes (I. Halvorsen System Design, Phoenix, AZ, USA) were divided into two compartments (each 30 cm L x 30 cm W x 25 cm H) with distinct floor textures (1 cm square wire mesh or 14 parallel horizontal bars spaced 1.25 cm apart) that were separated by an opaque plastic tunnel (7.5 cm L x 8 cm W x 7.5 cm H). The test environment was illuminated with light extending into the visible red frequency in accordance with prior studies from this laboratory.

Procedure – Place conditioning

The experiment had three phases: pre-conditioning (baseline), conditioning, and post-conditioning (retention). Each animal was always exposed to the same apparatus that was cleaned thoroughly between animals using diluted ammonia-based window cleaner (1:6 in water) (No Name Glass Cleaner with Ammonia, Loblaw Companies Ltd., Toronto, ON). **Pre-conditioning:** Each animal accessed the apparatus for 15 minutes per day for three days; a trained observer recorded time spent in each compartment and the tunnel. **Conditioning:** Animals received drug or saline injections on alternating days and were restricted to the respective drug- or vehicle-paired compartment for 30 minutes (tunnel was inaccessible). Groups were counterbalanced so that equal numbers of animals received drug in each compartment. **Post-conditioning:** Each animal accessed the apparatus in a drug-free state for 15 minutes per day for three days; a trained observer recorded time spent in each compartment and the tunnel.

Apparatus – Locomotor activity

Spontaneous locomotor activity was measured using computer-monitored photobeam boxes (I. Halvorsen System Design, Phoenix, AZ, USA) that consisted of Plexiglas test cages (43 cm L x 43 cm W x 30 cm H). A 12 x 12 array of horizontal infrared beams permitted recording of both horizontal and consecutive beam breaks. While horizontal activity includes any movement about the array and is an overall measure of activity, consecutive activity includes any repetitive movement (i.e., immediately consecutive beam breaks). Vertical activity (rearing behaviour) was measured using 12 additional beams 12 cm above, and parallel to, the floor. Drugs that induce stereotypy or alter the animals' rearing behaviour will be detected using consecutive or vertical measurements respectively, while an overall depiction of generalized activity is provided by horizontal measurements. Local time course data for each measure (horizontal, consecutive, and vertical) were automatically recorded during 5 minute intervals for the duration of the test period. The test environment was illuminated with light extending into the visible red frequency in accordance with prior studies in this laboratory.

Experimental designs

This chapter describes four separate experiments. Experiment 8 assessed whether systemic administration of MK-801 induces place conditioning. Experiment 9 assessed whether systemic administration of MK-801 affects conditioned place preference induced by systemic 7-OH-DPAT. Experiment 10 expanded upon a previous dose-response curve for place conditioning by 7-OH-DPAT; specifically, whether very low doses of 7-OH-DPAT may induce place conditioning. Experiment 11 assessed whether repeated systemic administration of MK-801 or 7-OH-DPAT, as described for place conditioning, also induces conditioned locomotor effects.

In experiments 8-10, animals were randomly assigned to an unbiased place conditioning design according to Table 6.1. In experiment 11, the treatment regimen followed that of the place conditioning procedure described above, except that animals were placed into a locomotor activity apparatus following drug, or home cages following saline.

Table 6.1. Experimental designs for experiments in Chapter 6. UPC = unbiased place conditioning. CLMA = conditioned locomotor activity.

Experiment	Group size	Treatments
8 - UPC	N=30; n=7-8	MK-801 (0.03, 0.06, or 0.13 mg kg^{-1}), or 7-OH-DPAT (5.0 mg kg^{-1})
9 - UPC	N=56; n=8	MK-801 (0.03, 0.06, or 0.13 mg kg^{-1}), 7-OH-DPAT (5.0 mg kg^{-1}), or combination of MK-801 + 7-OH-DPAT.
10 - UPC	N=23; n=7-8	7-OH-DPAT (0.005, 0.1, or 5.0 mg kg^{-1})
11 - CLMA	N=64; n=8	Same as experiment 9, plus SAL/SAL treatment

Statistical analyses

Place conditioning (or extinction) was determined by comparing time spent in the drug-paired compartment on pre-conditioning day 3 (baseline) with each day of post-conditioning (retention) using paired-samples t-tests. An increase or decrease in time spent in the drug-paired compartment reflects CPP or conditioned place aversion (CPA) respectively.

Locomotor activity data were analysed using a two-way (MK-801 x 7-OH-DPAT) between-subjects ANOVA on pre-conditioning (baseline) day 3, and post-conditioning (retention) day 1. Data from conditioning days were analysed using a three-way repeated measures ANOVA (MK-801 x 7-OH-DPAT x Days). For illustrative purposes, acute drug effects on conditioning day 1 are also shown; these data were analysed using a two-way (MK-801 x 7-OH-DPAT) between-subjects ANOVA. For all measures, in the absence of an MK-801 x 7-OH-DPAT interaction, the main effect of MK-801 was explored using a one-way between-subjects ANOVA followed by Newman-Keuls post-hoc tests. Alternately, in the presence of an MK-801 x 7-OH-DPAT interaction, effects of MK-801 were assessed using two separate one-way between-subjects ANOVA (in the presence or absence of 7-OH-DPAT) followed by Newman-Keuls post-hoc tests, while effects of 7-OH-DPAT at each dose of MK-801 were assessed using independent samples t-tests.

Local time-course data were analysed using a one-way between-subjects ANOVA across all treatments for each 5 minute interval. A significant F ratio

(P<0.05) on a single interval was followed by comparison of each drug with vehicle using a Newman-Keuls post-hoc test. Statistical analyses ($\alpha=0.05$) were completed using SPSS 11.5 (SPSS Inc. Chicago, IL, USA).

For each repeated measures experiment, Greenhouse-Geisser adjusted degrees of freedom have been used; because of this, degrees of freedom values may appear as non-integers.

Results

Experiment 8: MK-801 did not induce place conditioning

As shown in Figure 6.1(A), MK-801 (0.03, 0.06, 0.13 mg kg^{-1}) did not induce place conditioning (MK-801 0.03 mg kg^{-1}: t(7)=1.683, P=0.136; 0.06 mg kg^{-1}: t(6)=0.123, P=0.906; 0.13 mg kg^{-1}: t(7)=1.102, P=0.307) while 7-OH-DPAT (5.0 mg kg^{-1}) induced CPP (t(6)=3.719; P=0.010).

Experiment 9: MK-801 dose-dependently blocked 7-OH-DPAT CPP

As described above, 7-OH-DPAT induced a CPP (t(6)=3.719, P=0.010). Co-administration of 0.03 mg kg^{-1} MK-801 with 7-OH-DPAT also induced CPP (t(7)=3.453, P=0.011). These results are shown in Figure 6.1(B). These preferences extinguished the next day (7-OH-DPAT: t(6)=1.036, P=0.340; MK-801+7-OH-DPAT: t(7)=2.091, P=0.075) and this is shown in Figures 6.2(A) and 6.2(B) respectively. Co-administration of higher doses of MK-801 with 7-OH-DPAT did not induce place conditioning (MK-801 0.06 mg kg^{-1}: t(6)=1.072, P=0.325; 0.13 mg kg^{-1}: t(7)=0.610, P=0.561) and these results are also shown in Figure 6.1(B).

Experiment 10: Low doses of 7-OH-DPAT did not induce place conditioning

As shown in Figure 6.3, 7-OH-DPAT (0.005 or 0.01 mg kg^{-1}) did not induce place conditioning (0.005 mg kg^{-1}: t(7)=0.342, P= 0.742; 0.01 mg kg^{-1}:

$t(7)=0.129$, $P=0.901$) while 7-OH-DPAT (5.0 mg kg^{-1}) induced CPP ($t(6)=3.719$; $P=0.010$).

Figure 6.1. Effects of MK-801 on place conditioning (A; n=7-8) and on CPP induced by 7-OH-DPAT (5.0 mg kg^{-1}) (B; n=7-8). Post-conditioning data are from the first day of retention testing. Significant difference (*) from pre-conditioning at $P<0.05$ (mean ± SEM).

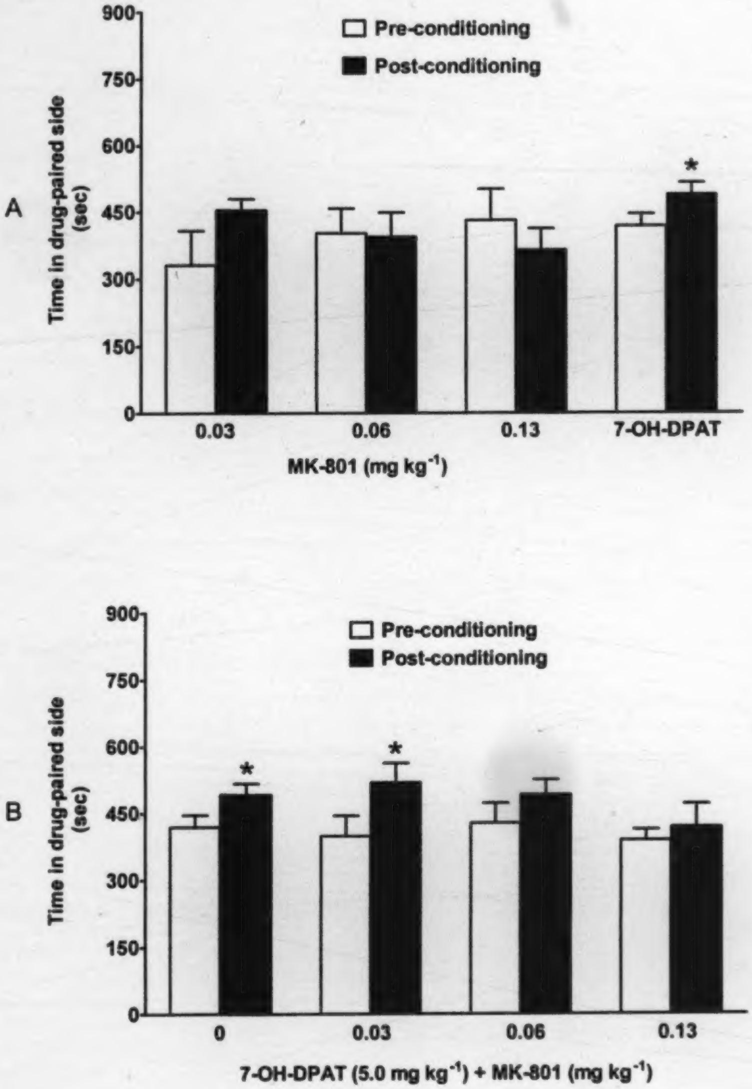


Figure 6.2. Extinction of CPP induced by 7-OH-DPAT (A; n=7) and co-administration of 7-OH-DPAT and 0.03 mg kg^{-1} MK-801 (B; n=8). Significant difference (*) from pre-conditioning at $P<0.05$ (mean ± SEM).

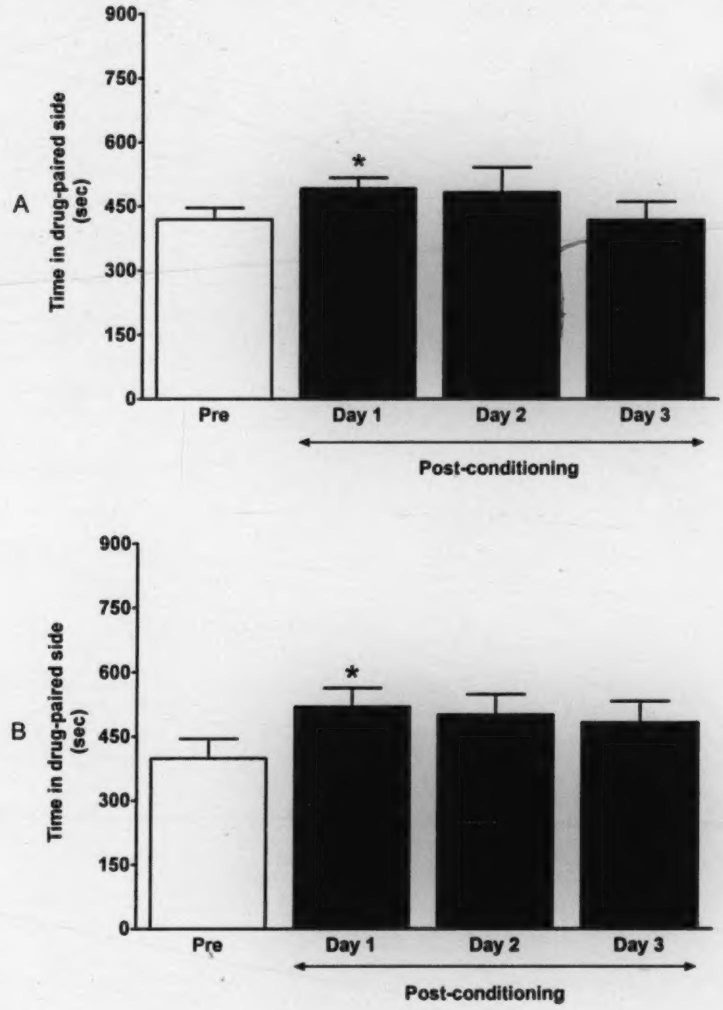
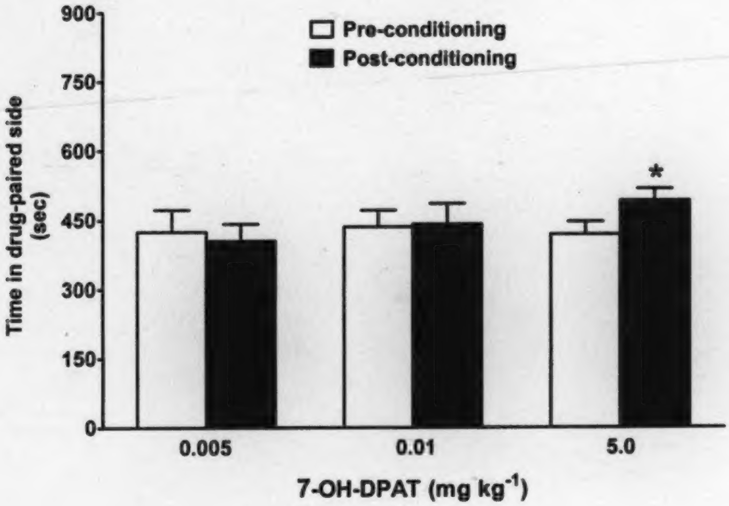


Figure 6.3. Low doses of 7-OH-DPAT did not induce place conditioning (n=7-8). Post-conditioning data are from the first day of retention testing. Significant difference (*) from pre-conditioning at P<0.05 (mean ± SEM).



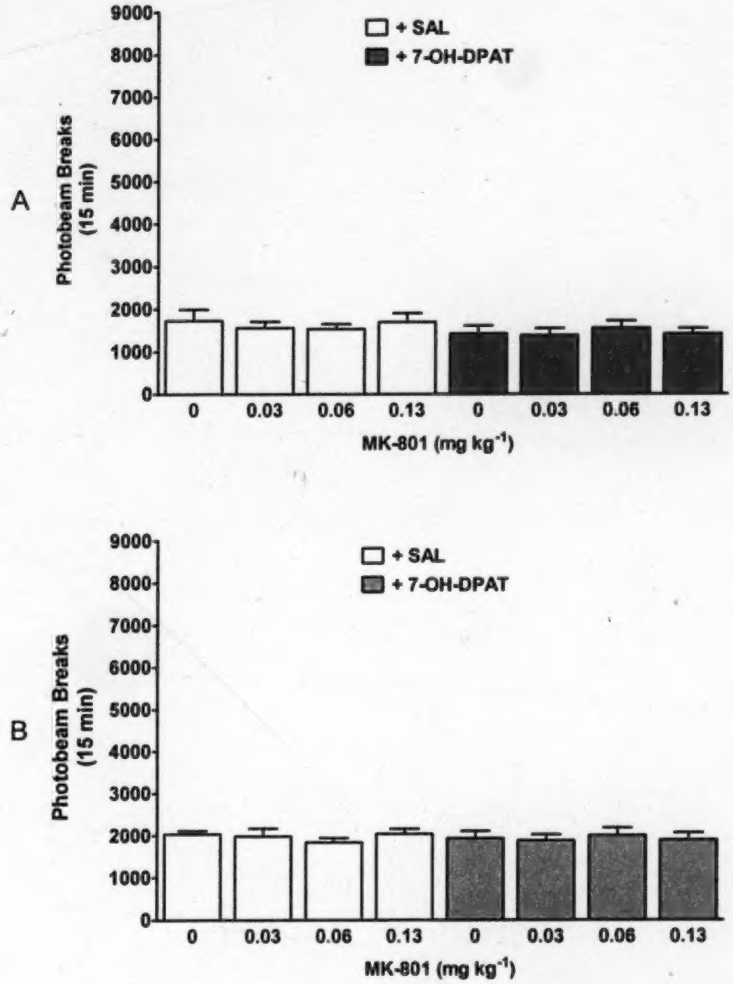
Experiment 11: Repeated systemic administration of MK-801 or 7-OH-DPAT, as needed for place conditioning, did not induce conditioned locomotor effects

Horizontal activity

All groups showed equivalent activity counts on pre-conditioning day 3 (Figure 6.4(A)). There were no main effects of MK-801 (F(3,56)=0.127, P=0.944) or 7-OH-DPAT (F(1,56)=2.112, P=0.152), nor significant MK-801 x 7-OH-DPAT interaction (F(3,56)=0.321, P=0.810). In addition, all groups showed equivalent activity on post-conditioning day 1 (retention) (Figure 6.4(B)). There were no main effects of MK-801 (F(3,56)=0.082, P=0.970) or 7-OH-DPAT (F(1,56)=0.179, P=0.674), nor significant MK-801 x 7-OH-DPAT interaction (F(3,56)=0.417, P=0.741).

Analysis of conditioning days revealed significant main effects of MK-801 (F(3,56)=12.771, P<0.001) and 7-OH-DPAT (F(1,56)=61.532, P<0.001), but no MK-801 x 7-OH-DPAT interaction (F(3,56)=1.544, P=0.213). There was a main effect of Days (F(1.786,112)=4.656, P=0.015), and significant 7-OH-DPAT x Days interaction (F(1.786,112)=3.437, P=0.041). However, there was no MK-801 x Days interaction (F(5.359,112)=1.254, P=0.288) nor MK-801 x 7-OH-DPAT x Days interaction (F(5.359,112)=1.387, P=0.232).

Figure 6.4. Effects of MK-801 and 7-OH-DPAT (5.0 mg kg^{-1}) on total horizontal activity on pre-conditioning day 3 (A) and post-conditioning day 1 (B) (mean ± SEM; n=8).



For illustrative purposes, acute drug effects on conditioning day 1 are shown in Figure 6.5(A). On this day, there were main effects of MK-801 (F(3,56)=6.750, P=0.001) and 7-OH-DPAT (F(1,56)=24.265, P<0.001), but no MK-801 x 7-OH-DPAT interaction (F(3,56)=0.094, P=0.963). Further analysis of the main effect of MK-801 (Figure 6.6) showed that MK-801 (0.13 mg kg^{-1}) increased horizontal activity compared to control treatment (Newman Keuls P<0.05).

Also for illustrative purposes, the local time course of drug effects on horizontal locomotor activity are shown in Figure 6.5(B). The co-administration of MK-801 (0.13 mg kg^{-1}) and 7-OH-DPAT (5.0 mg kg^{-1}) increased horizontal activity relative to control treatment at 10, 15, 20, 25, and 30 minutes (Newman Keuls P<0.05).

Consecutive activity

All groups showed equivalent activity counts on pre-conditioning day 3 (Figure 6.7(A)). There were no main effects of MK-801 (F(3,56)=0.922, P=0.436) or 7-OH-DPAT (F(1,56)=0.025, P=0.874), nor significant MK-801 x 7-OH-DPAT interaction (F(3,56)=0.166, P=0.919). In addition, all groups showed equivalent activity on post-conditioning day 1 (retention) (Figure 6.7(B)). There were no main effects of MK-801 (F(3,56)=0.171, P=0.916) or 7-OH-DPAT (F(1,56)=3.546, P=0.065), nor significant MK-801 x 7-OH-DPAT interaction (F(3,56)=0.049, P=0.986).

Figure 6.5. Effects of MK-801 and 7-OH-DPAT (5.0 mg kg^{-1}) on total horizontal activity on conditioning day 1 (A) (mean ± SEM; n=8). The local time course data of horizontal activity on conditioning day 1 (B) are shown as means (SEM omitted for clarity). Significant difference (*) from control at P<0.05.

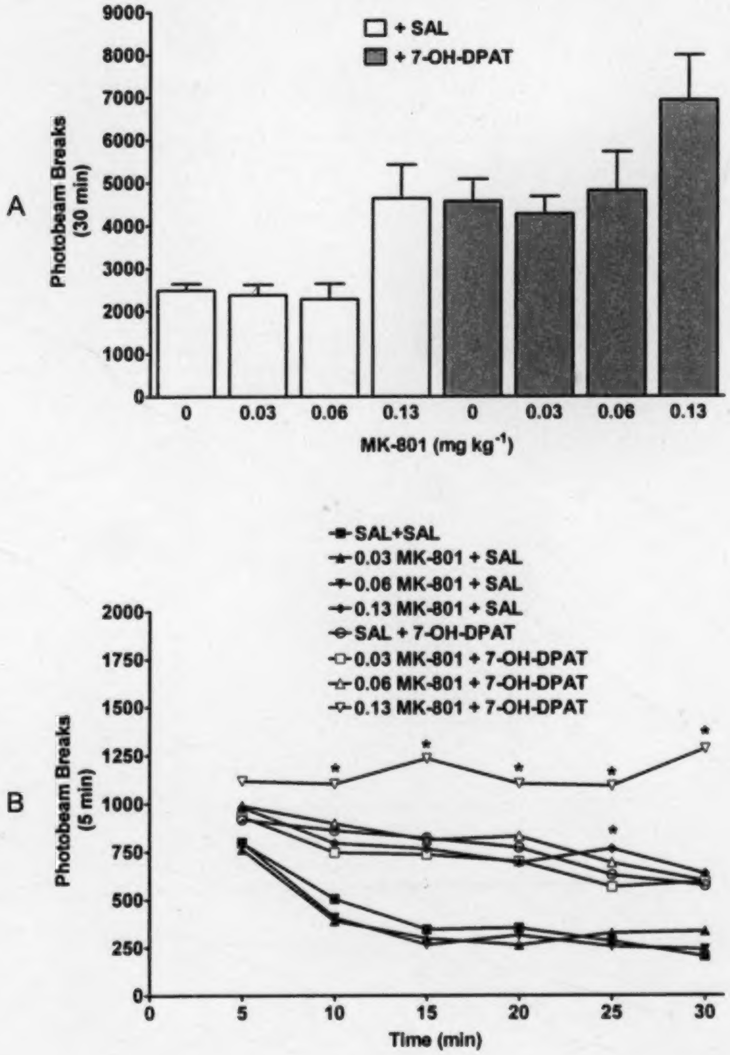


Figure 6.6. Further analysis of the main effect of MK-801 on total horizontal locomotor activity on conditioning day 1 (mean ± SEM; n=16). Significant difference (*) from control at P<0.05.

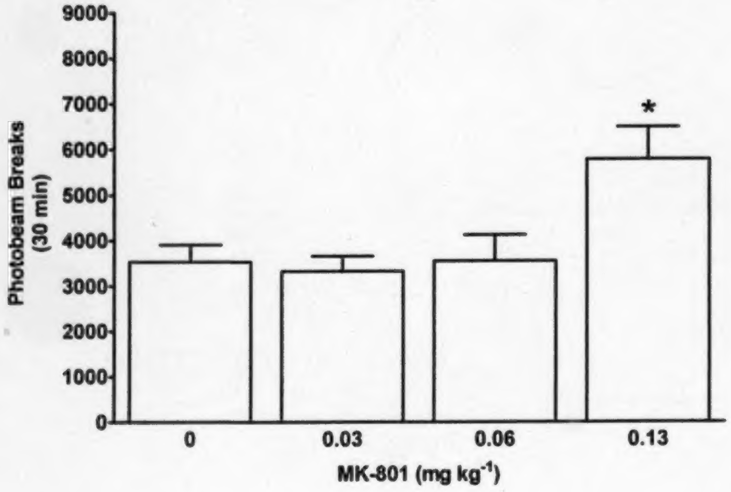
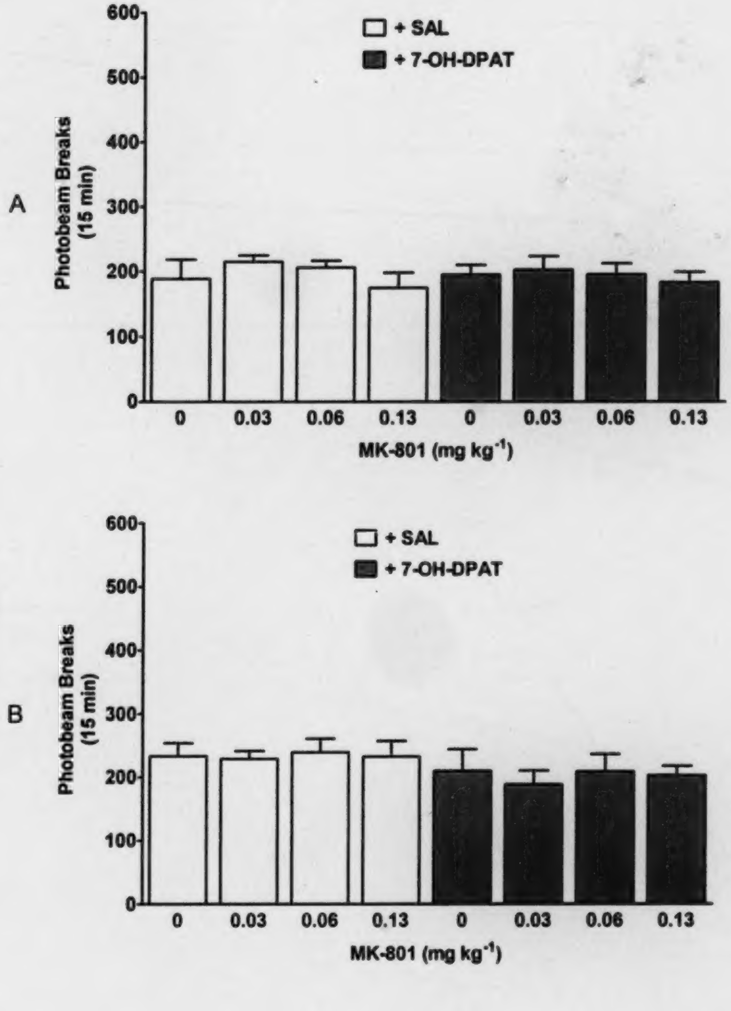


Figure 6.7. Effects of MK-801 and 7-OH-DPAT (5.0 mg kg^{-1}) on total consecutive activity on pre-conditioning day 3 (A) and post-conditioning day 1 (B) (mean ± SEM; n=8).

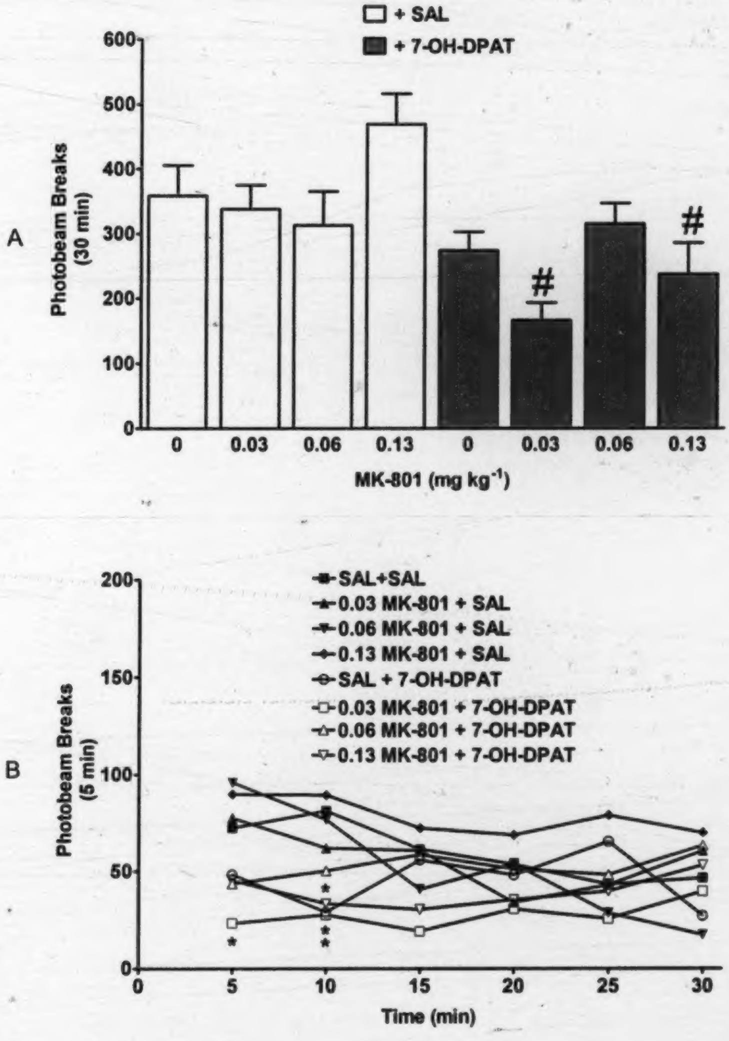


Analysis of conditioning days revealed a significant main effect of 7-OH-DPAT (F(1,56)=12.875, P=0.001), but not of MK-801 (F(3,56)=0.517, P=0.673), and no MK-801 x 7-OH-DPAT interaction (F(3,56)=1.493, P=0.226) on consecutive activity. There was a main effect of Days (F(1.884,112)=5.380, P=0.007), and significant 7-OH-DPAT x Days interaction (F(1.884,112)=3.410, P=0.039) and MK-801 x Days interaction (F(5.653,112)=2.678, P=0.021). However, there was no MK-801 x 7-OH-DPAT x Days interaction (F(5.653,112)=1.086, P=0.375).

For illustrative purposes, acute drug effects on conditioning day 1 are shown in Figure 6.8(A). On this day, there was a main effect of 7-OH-DPAT (F(1,56)=17.551, P<0.001), but not of MK-801 (F(3,56)=2.101, P=0.110), and significant MK-801 x 7-OH-DPAT interaction (F(3,56)=3.099, P=0.034). Further tests revealed MK-801 was ineffective alone (F(3,28)=2.234, P=0.106), but was effective in the presence of 7-OH-DPAT (F(3,28)=3.227, P=0.037); however there were no significant differences among groups (Newman-Keuls P>0.05). 7-OH-DPAT alone was ineffective but decreased consecutive activity in the presence of MK-801 (0.03 mg kg^{-1}: t(14)=3.731, P=0.002; 0.13 mg kg^{-1}: t(14)=3.432, P=0.004 but not 0.06 mg kg^{-1}).

Also for illustrative purposes, the local time course of drug effects on consecutive locomotor activity are shown in Figure 6.8(B). Co-administration of MK-801 (0.03 mg kg^{-1}) and 7-OH-DPAT (5.0 mg kg^{-1}) decreased this measure relative to control treatment at 5 minutes; in addition, 7-OH-DPAT or co-administration of 7-OH-DPAT with MK-801 (0.03 or 0.13 mg kg^{-1}) decreased consecutive activity relative to control treatment at 10 minutes (Newman-Keuls $P<0.05$).

Figure 6.8. Effects of MK-801 and 7-OH-DPAT (5.0 mg kg^{-1}) on total consecutive activity on conditioning day 1 (A) (mean ± SEM; n=8). Significant difference (#) from that dose of MK-801 alone at $P<0.05$. The local time course data of consecutive activity on conditioning day 1 (B) are shown as means (SEM omitted for clarity). Significant difference (*) from control at $P<0.05$.



Vertical activity

All groups showed equivalent activity counts on pre-conditioning day 3 (Figure 6.9(A)). There were no main effects of MK-801 (F(3,56)=0.653, P=0.585) or 7-OH-DPAT (F(1,56)=3.081, P=0.085), nor significant MK-801 x 7-OH-DPAT interaction (F(3,56)=1.535, P=0.215). In addition, all groups showed equivalent activity on post-conditioning day 1 (retention) (Figure 6.9(B)). There were no main effects of MK-801 (F(3,56)=0.868, P=0.463) or 7-OH-DPAT (F(1,56)=1.998, P=0.163), nor significant MK-801 x 7-OH-DPAT interaction (F(3,56)=0.592, P=0.623).

Analysis of conditioning days revealed a significant main effect of 7-OH-DPAT (F(1,56)=99.873, P<0.001), but not of MK-801 (F(3,56)=2.337, P=0.083), and there was no MK-801 x 7-OH-DPAT interaction (F(3,56)=2.103, P=0.110). There was no main effect of Days (F(1.920,112)=0.338, P=0.705), no 7-OH-DPAT x Days interaction (F(1.920,112)=0.208, P=0.804), no MK-801 x Days interaction (F(5.761,112)=1.264, P=0.281), nor MK-801 x 7-OH-DPAT x Days interaction (F(5.761,112)=1.350, P=0.243).

For illustrative purposes, acute drug effects on conditioning day 1 are shown in Figure 6.10(A). On this day, there were main effects of MK-801 (F(3,56)=5.034, P=0.004) and 7-OH-DPAT (F(1,56)=78.460, P<0.001), and significant MK-801 x 7-OH-DPAT interaction (F(3,56)=4.433, P=0.007). Further tests on the interaction term revealed that MK-801 was without effect in the presence of 7-OH-DPAT (F(3,28)=1.217, P=0.321); however, in the absence of 7-

OH-DPAT, there was a main effect MK-801 ($F(3,28)=4.748$, $P=0.008$). Post-hoc tests revealed that MK-801 (0.06 or 0.13 mg kg^{-1}) decreased vertical activity relative to control treatment (Newman-Keuls $P<0.05$). 7-OH-DPAT decreased vertical activity alone ($t(14)=4.812$, $P<0.001$) and in the presence of each dose of MK-801 (0.03 mg kg^{-1}: $t(14)=4.409$, $P=0.001$; 0.06 mg kg^{-1}: $t(14)=6.107$, $P<0.001$; 0.13 mg kg^{-1}: $t(14)=4.896$, $P<0.001$).

Also for illustrative purposes, the local time course of drug effects on vertical locomotor activity are shown in Figure 6.10(B). All drug treatments decreased vertical activity relative to control treatment at 5 minutes. In addition, 7-OH-DPAT alone decreased this measure at 10 and 20 minutes, while co-administration of all doses of MK-801 with 7-OH-DPAT decreased vertical activity relative to control treatment at 10, 15, and 20 minutes (Newman-Keuls $P<0.05$).

Figure 6.9. Effects of MK-801 and 7-OH-DPAT (5.0 mg kg^{-1}) on total vertical activity on pre-conditioning day 3 (A) and post-conditioning day 1 (B) (mean ± SEM; n=8).

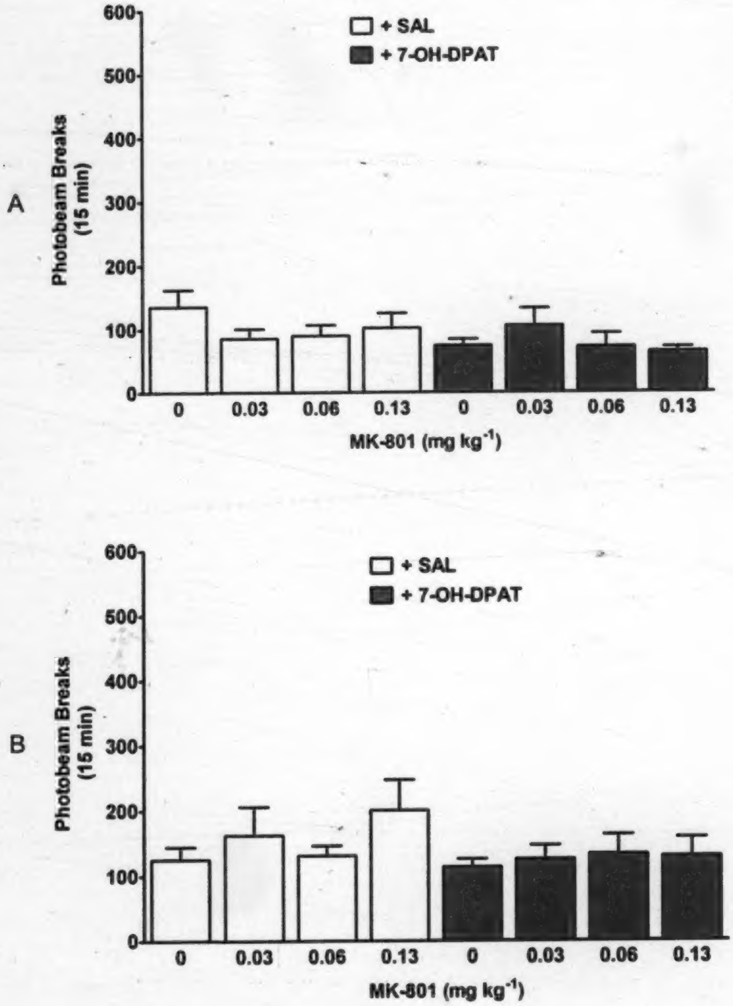
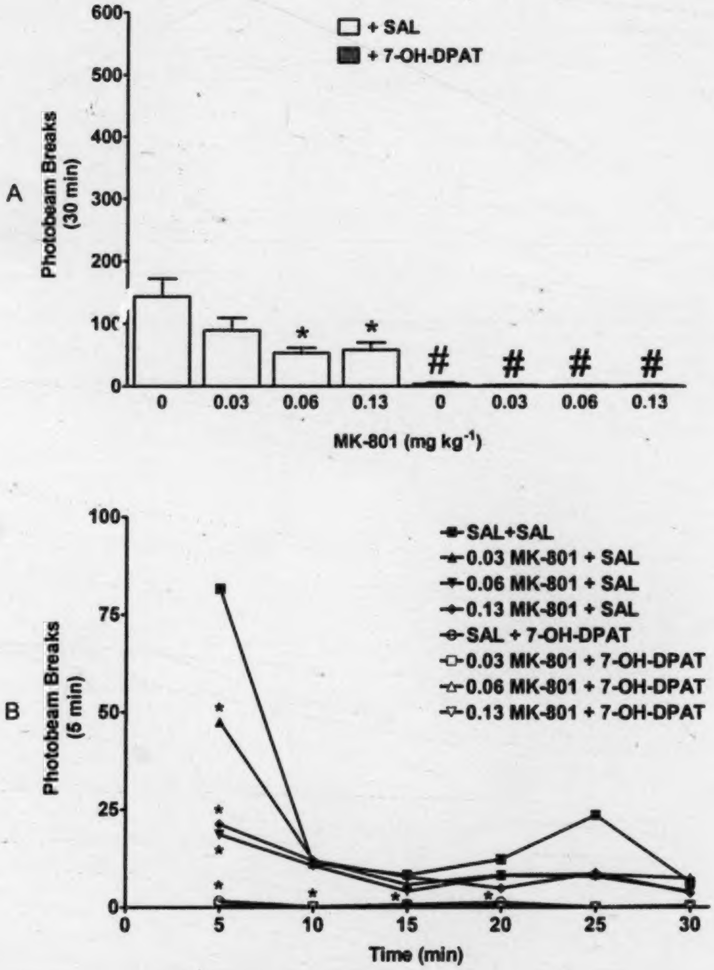


Figure 6.10. Effects of MK-801 and 7-OH-DPAT (5.0 mg kg^{-1}) on total vertical activity on conditioning day 1 (A) (mean ± SEM; n=8). Significant difference (*) from control at $P<0.05$. Significant difference (#) from that dose of MK-801 alone at $P<0.05$. The local time course data of vertical activity on conditioning day 1 (B) are shown as means (SEM omitted for clarity). Significant difference (*) from control at $P<0.05$.



Discussion

The main findings of this chapter are listed below. Each finding will be briefly discussed in turn in this chapter. Discussion that relates the separate studies to each other is reserved for Chapter 7 (General Discussion).

- MK-801 (0.03-0.13 mg kg^{-1} IP 15 min prior) did not induce place conditioning (Experiment 8).
- 7-OH-DPAT (5.0 mg kg^{-1} SC 15 min prior) induced conditioned place preference that was blocked by co-administration of MK-801 (0.06 or 0.13 mg kg^{-1}, but not 0.03 mg kg^{-1}, IP 15 min prior) during conditioning (Experiment 9).
- 7-OH-DPAT (0.005 or 0.01 mg kg^{-1} SC 15 min prior) did not induce place conditioning (Experiment 10).
- Repeated administration of MK-801 (0.03-0.13 mg kg^{-1} IP 15 min prior) or 7-OH-DPAT (5.0 mg kg^{-1} SC 15 min prior) did not induce conditioned locomotor effects (Experiment 11).

MK-801 (0.03-0.13 mg kg^{-1} IP 15 min prior) did not induce place conditioning

The lack of effect of MK-801 in place conditioning agrees with numerous prior reports (Sufka 1994; Hoffman 1994; Steinpreis et al. 1995; Tzschentke and Schmidt 1995; Kim et al. 1996; Kim and Jang 1997; Tzschentke and Schmidt 1998; Sukhotina et al. 1998; Ribeiro Do Couto et al. 2004). However, some studies show that MK-801 may induce a CPP (Layer et al. 1993a; Papp and Moryl 1994; Sufka 1994; Hoffman 1994; Steinpreis et al. 1995; Papp et al. 1996; Del Pozo et al. 1996; Sukhotina et al. 1998; Suzuki et al. 1999; Panos et al. 1999; Biała and Kotlińska 1999). Procedural and apparatus differences may account for these inconsistencies. For example, it may be that place conditioning effects of MK-801 may be detected using a biased design, in which animals receive the conditioning drug in the environment that is initially least-preferred. The present study used an unbiased place conditioning procedure that balances initial preferences among groups.

7-OH-DPAT (5.0 mg kg^{-1} SC 15 min prior) induced conditioned place preference that was blocked by MK-801 (0.06 or 0.13 mg kg^{-1}, but not 0.03 mg kg^{-1}, IP 15 min prior)

7-OH-DPAT (5.0 mg kg^{-1}) induced a CPP when administered 15 minutes prior to conditioning, in accord with prior studies that used a delay (5.0 mg kg^{-1} 30 min prior Mallet and Beninger 1994; 4.0 mg kg^{-1} 30 min prior Chaperon and Thiébot 1996; 0.25 mg kg^{-1} 10 min prior Kling-Petersen et al. 1995b; 5.0 mg kg^{-1} 15 min prior Biondo 2002). Based on receptor affinity (Lévesque et al. 1992), it has been previously suggested that 7-OH-DPAT may induce CPP by stimulation of D_2 DA receptors (e.g., Biondo 2002). Similarly, other D_2 DA receptor agonists (such as bromocriptine and quinpirole) may also induce CPP (Hoffman et al. 1988; White et al. 1991). A relatively high dose of (+)-7-OH-DPAT (0.328 mg

kg^{-1}) also induces sniffing behaviour, a marker of postsynaptic D_2 DA receptor effects, while lower doses do not (Damsma et al. 1993).

MK-801 inhibited acquisition of CPP induced by the selective $D_{2/3}$ DA receptor agonist 7-OH-DPAT. Since MK-801 alone was ineffective, attenuation of the CPP induced by systemic 7-OH-DPAT cannot be due to additive effects. Similar doses of MK-801 may also inhibit or attenuate acquisition of CPP induced by cocaine or methamphetamine (Cervo and Samanin 1995; Kim et al. 1996; Kim and Jang 1997). Similar effects have been found with another noncompetitive NMDA antagonist memantine (Kotlińska and Biała 2000). In contrast, MK-801 may not affect acquisition of CPP induced by amphetamine (Hoffman 1994); however, in that study MK-801 alone induced CPP.

In the present study, it is unlikely that MK-801 directly reduced the reinforcing effect of 7-OH-DPAT, as MK-801 has been shown to facilitate brain stimulation reward (Corbett 1989; Herberg and Rose 1989; Carlezon and Wise 1993; Carlezon and Wise 1996; Olds 1996; Cabeza de Vaca and Carr 1998; Sukhotina et al. 1999; De Vry et al. 2001; Sundstrom et al. 2002; Kenny et al. 2003). Rather, it is possible that MK-801 may nonspecifically impair the process of associative learning necessary for the development of place conditioning – it has been shown previously that NMDA receptor antagonists may impair both associative and non-associative forms of learning (e.g., Riters and Bingman 1994; Thompson and Disterhoft 1997).

It has also been shown previously that NMDA antagonists like MK-801 may induce state-dependent operant learning for food reward (Jackson et al. 1992). However, it is not likely that the blockade of 7-OH-DPAT CPP by MK-801 is due to state-dependency; MK-801 has blocked morphine-induced CPP, but did not make the retention of this CPP state-dependent (Tzschentke and Schmidt 1997).

7-OH-DPAT (0.005 or 0.01 mg kg^{-1} SC 15 min prior) did not induce place conditioning

Some reports suggest that 7-OH-DPAT may be ineffective in place conditioning (0.003-5.0 mg kg^{-1} Khroyan et al. 1995; 0.1, 0.25, and 0.5 mg kg^{-1} Rodríguez De Fonseca et al. 1995). In the present study, 7-OH-DPAT (0.005 or 0.01 mg kg^{-1}) also failed to induce place conditioning. This result contrasts previous reports of CPA induced by low doses of 7-OH-DPAT (0.004 or 0.008 mg kg^{-1} Chaperon and Thiébot 1996; 1 or 3 mg kg^{-1} in mice Kamei and Ohsawa 1996; 0.05 or 0.1 mg kg^{-1} Gyertyán and Gál 2003). As the selective D_3 DA agonist PD-128907 and D_3 DA partial agonist BP-897 may also induce CPA (Gyertyán and Gál 2003), the potentially aversive properties of low doses of 7-OH-DPAT may be attributable to stimulation of D_3 DA receptors. As described in previous chapters, there is other evidence for inhibitory postsynaptic D_3 DA receptors (Waters et al. 1993; Svensson et al. 1994b,c; Mattingly et al. 1996; Depoortere et al. 1996; Zarandi 1998).

Repeated administration of MK-801 (0.03-0.13 mg kg^{-1} IP 15 min prior) or 7-OH-DPAT (5.0 mg kg^{-1} SC 15 min prior) did not induce conditioned locomotor effects

Psychostimulants (e.g., amphetamine) induce hyperactivity that can be reinstated by the drug-paired environment (Beninger and Hahn 1983). In the present study, acute administration of MK-801 or a "high dose" of 7-OH-DPAT induced hyperactivity, in accord with prior reports (see Biondo 2002). That repeated administration of either drug does not induce conditioned locomotor hyperactivity is surprising. This lack of effect is not likely due to latent inhibition, as habituation to the test environment may not affect conditioned locomotor responses to cocaine (Martin-Iverson and Reimer 1996).

Conclusion

In this study, systemic NMDA receptor blockade by MK-801 did not induce place conditioning, but inhibited the acquisition of CPP induced by the $D_{2/3}$ DA receptor agonist 7-OH-DPAT. Although acute administration of either compound induced hyperactivity, repeated administration did not induce any conditioned locomotor effects. These findings suggest that acquisition of conditioned reinforcement elicited by stimulation of D_2 DA receptors can be reversed by simultaneous NMDA receptor blockade. In addition, D_2 DA receptor stimulation induces place conditioning independently of any conditioned changes in locomotor activity.

Chapter 7. General discussion and conclusions

Interactions between DA and Glu in the mesolimbic system of the brain may affect mechanisms of reward and reinforcement. As such, it has been previously suggested that abnormal DA-Glu function may play a role in human disorders of motivation, and may be related to the pathophysiology of schizophrenia and drug abuse. The purpose of the present set of studies was to characterize further the behavioural effects of drugs that affect DA or Glu using measurements of motivated behaviour in rodents. This was accomplished using systemic administration of ligands targeting specific DA or Glu receptors, or direct application of such ligands into the NAS shell subregion. Drug effects and drug-drug interactions were assessed by measuring 1) spontaneous locomotor activity; 2) rate-frequency thresholds in brain stimulation reward; and 3) place conditioning. While the majority of prior DA-Glu interaction studies have used nonselective DA agonists such as amphetamine and cocaine (see Chapter 2), the primary focus of this thesis was the potential interaction between D_2-like DA receptor stimulation and NMDA or AMPA/kainate receptor antagonism.

The present set of studies employed the following compounds: the noncompetitive NMDA receptor antagonist MK-801; the AMPA/kainate receptor antagonist NBQX; the $D_{2/3}$ DA receptor agonist 7-OH-DPAT; and the nonspecific DA receptor agonist apomorphine. It is noteworthy that in the present set of studies, apomorphine was always given systemically at a dose that is recognized as selective for the stimulation of D_2-like DA autoreceptors (see page 24).

The main findings of each part of the present set of studies are briefly described below, followed by the relevant discussion for each topic.

Glu receptor antagonism

The main findings for this section are as follows:

- MK-801 (0.13 mg kg^{-1}) induced hyperactivity (Experiments 1, 2).
- MK-801 (0.03 mg kg^{-1} or 0.13 mg kg^{-1}) decreased BSR rate-frequency thresholds, indicating increased reward, without altering response performance (Experiments 4, 5).
- MK-801 (0.66 μg intra-NAS shell) induced hyperactivity (Experiment 3).
- MK-801 (0.66 μg intra-NAS shell) decreased BSR rate-frequency thresholds, indicating increased reward, without altering response performance (Experiment 6).
- MK-801 (0.03, 0.06, or 0.13 mg kg^{-1}) did not induce place conditioning (Experiment 8).
- Repeated administration of MK-801 (0.03, 0.06, 0.13 mg kg^{-1}) did not induce conditioned locomotor effects (Experiment 11).
- Intra-NAS shell NBQX (0.5 μg) did not affect BSR rate-frequency thresholds and did not alter response performance (Experiment 7).

The differential interactions between DA agonists and NMDA vs. AMPA/kainate receptor antagonists are intriguing. Recall that NMDA receptors are ligand-gated ion channels permeable to cations such as calcium, and at resting membrane potentials, NMDA receptors are inactive due to a voltage-dependent blockade of the channel by magnesium ions (Mg^{2+}). Alternatively, AMPA and kainate receptors are non-NMDA Glu receptors that function as cation channels to influence membrane polarization. Currently there are few antagonists that can distinguish between AMPA and kainate receptors; NBQX (Sheardown et al. 1990) may be the most selective AMPA receptor antagonist currently available. It seems logical to presume that endogenous Glu would simultaneously stimulate both NMDA and AMPA receptors; however, without membrane depolarization, the NMDA receptor is inactive. Therefore, activation of AMPA receptors may provide the membrane polarization necessary for removal of the Mg^{2+} blockade of the NMDA receptor. If this hypothesis is true, then blockade of AMPA receptors should produce similar effects to those observed with NMDA receptor blockade alone.

However, this theory is generally not supported by the behavioural literature. Whereas NMDA receptor antagonists such as MK-801 induce robust hyperactivity and facilitate BSR (see Chapters 3 and 4), administration of AMPA/kainate receptor antagonists alone may be inactive in naïve rats or mice (Danysz et al. 1994; Starr and Starr 1994; Bubser et al. 1995; Kaddis et al. 1995; Li et al. 1997; Choi et al. 2000; Biondo 2002). It appears that effects of

AMPA/kainate receptor antagonists and NMDA receptor antagonists, when given alone, are not equivalent in the context of reward-related behaviour. The reason why NMDA receptor antagonists are effective in this context, but AMPA/kainate receptor antagonists alone are not, is unclear. It is likely that NMDA receptor function is enabled, in this context, by membrane depolarization produced independently of AMPA receptor stimulation. This possibility is expanded upon below (see page 231).

The stimulatory effect of NMDA receptor blockade may be due to activation of the mesolimbic DA system, as MK-801 has been shown to increase DA turnover in the NAS (Bristow et al. 1993), when turnover is defined as the ratio of metabolites to DA: [DOPAC+HVA]/[DA]. However, this effect is controversial; Druhan et al. (1996) reported that MK-801 alone did not affect extracellular DA in the NAS, nor did MK-801 influence effects of amphetamine or apomorphine on DA in the NAS. It is noteworthy however that in that study, MK-801 dose-dependently increased DA metabolites in the NAS (Druhan et al. 1996).

The mechanism for the effect of MK-801 on DA in the NAS is unclear. Antagonism of NMDA receptors in the NAS may inhibit NAS GABA-containing interneurons that feedback to the VTA; disinhibition (i.e., activation) of the VTA would in turn stimulate DA release in the NAS. There is also further evidence that MK-801 effects may be DA-dependent; the stimulant effect has been blocked by D_2-like DA receptor antagonists including raclopride, YM 09151-2, sulpiride, or haloperidol, and may or may not be blocked by the D_1-like DA receptor antagonist SCH 23390 (Dall'Olio et al. 1992; Ouagazzal et al. 1993; Adriani et al. 1998). In general, it is likely that the mechanism responsible for the stimulatory effects of NMDA receptor blockade extends beyond DA, and may involve the inhibitory neurotransmitter GABA. The possibility for involvement of systems beyond DA is supported by studies that have reported robust stimulant effects of MK-801 in monoamine-depleted or DA-deficient mice (Carlsson and Carlsson 1989; Svensson et al. 1992; Chartoff et al. 2005).

Repeated administration of some compounds, such as psychostimulants, may induce progressively greater behavioural effects, a process referred to as behavioural sensitization. In the present study (see Chapter 6), MK-801 did not induce locomotor sensitization after 3 drug exposures; this is generally consistent with prior studies (Oles et al. 1990; Wolf and Khansa 1991; Dall'Olio et al. 1992; Wolf et al. 1993; Tzschentke and Schmidt 1997). In the context of behavioural sensitization and Glu receptors, it is noteworthy that AMPA/kainate receptor antagonists may inhibit locomotion and behavioural sensitization or conditioned reinforcement induced by repeated administration of some drugs of abuse, such as amphetamine and cocaine (Karler et al. 1991; Burns et al. 1994; Karler et al. 1994; Li et al. 1997). This is likely related to studies showing that repeated administration of psychomotor stimulants may alter the expression of AMPA receptor subunits in the NAS (Lu et al. 1997; Churchill et al. 1999; Kelz et al. 1999; Lu and Wolf 1999).

DA receptor stimulation

The main findings for this section are as follows:

- 7-OH-DPAT (0.03 mg kg^{-1}) induced hypoactivity (Experiment 2).
- 7-OH-DPAT (0.03 mg kg^{-1}) increased BSR rate-frequency thresholds, indicating decreased reward, and may or may not decrease response performance (Experiment 4).
- Intra-NAS shell 7-OH-DPAT (5.0 μg) did not affect spontaneous locomotor activity (Experiment 3).
- Intra-NAS shell 7-OH-DPAT (5.0 μg) may or may not increase BSR rate-frequency thresholds, but decreased response performance (Experiments 6, 7).
- 7-OH-DPAT (0.005 or 0.01 mg kg^{-1}) did not induce place conditioning (Experiment 10).
- 7-OH-DPAT (5.0 mg kg^{-1}) induced conditioned place preference (Experiments 8, 9).
- Repeated administration of 7-OH-DPAT (5.0 mg kg^{-1}) did not induce conditioned locomotor effects (Experiment 11).
- Apomorphine (0.05 mg kg^{-1}) induced hypoactivity (Experiment 1).
- Apomorphine (0.05 mg kg^{-1}) increased BSR rate-frequency thresholds, indicating decreased reward, without altering response performance (Experiment 5).

It is clear that DA is intricately associated with reward processes. However, the precise role of DA in motivation remains controversial. For example, contemporary theories by Schultz (Schultz 1998; Schultz 2000; Tobler et al. 2005) suggest that changes in DA transmission may be a result of the discrepancy between reward prediction and occurrence, rather than as a result of reward per se. In addition, both Ungless (2004) and Salamone et al. (2005) have recently outlined evidence showing that DA transmission is stimulated by both novel and conditioned stimuli, and that DA modulates a variety of behaviours not directly linked to reward, such as aversion and sensimotor gating. The role of DA is therefore not simple; however, in the context of BSR, it is clear that electrical stimulation of the VTA increases DA release in the NAS, and that DA may play a role in mediating the reinforcement of this behaviour (Gratton et al. 1988; Phillips et al. 1989; Fiorino et al. 1993). In addition, it is noteworthy that recent evidence has suggested a revised role for DA in BSR (Garris et al. 1999). However, a detailed account of the contribution of DA to reinforcement is beyond the scope of this discussion; the present set of experiments described the effects of only two DA receptor agonists.

This thesis has used two DA agonists with clear differences in mechanisms of action. As such, the remainder of this section is divided into two parts to discuss the behavioural effects and mechanisms of 7-OH-DPAT and apomorphine respectively.

7-OH-DPAT generally demonstrates biphasic effects in studies of behaviour. Higher doses (>2 mg kg^{-1}) generally induce conditioned place preferences and hyperactivity, while lower doses reduce activity and may induce conditioned place aversions (Biondo 2002; see Chapter 6). It has been previously proposed that, based on correlation with binding affinities, low doses may be linked to activation of D_3 DA receptors and inhibition of behaviour, while higher doses progressively activate D_2 DA receptors to stimulate behaviour. This would agree with the finding that another D_3 DA receptor agonist, PD 128,907, also reduces spontaneous locomotor activity in rats (Bristow et al. 1996). In addition, Ireland et al. (2005) have recently shown that another $D_{2/3}$ DA agonist, quinelorane, induced hypoactivity at low doses that corresponded to increased neuronal activity in the NAS, as measured using pharmacological MRI. Higher doses of quinelorane induced hyperactivity that corresponded to increased activation of the CP and GP. As the NAS demonstrates high expression of D_3 DA receptors, and the CP and GP have more D_2 DA receptors, hypoactivity induced by low doses is likely mediated by D_3 DA receptors in the NAS (Ireland et al. 2005).

The direct application of 7-OH-DPAT into the brain has yielded valuable information that validates the NAS shell as an important brain region that contributes to its effects on reward. However, it is difficult to compare intra-NAS results with those obtained from peripheral injection studies. For example, the lack of effect of intra-NAS 7-OH-DPAT on spontaneous exploratory activity

(Chapter 3) is consistent with its lack of effectiveness in VTA brain stimulation reward (Chapter 5; Choi 2000) and its inability to induce place conditioning (Biondo 2002). However, when given systemically, 7-OH-DPAT clearly reduces spontaneous activity and decreases BSR, and may induce CPA at lower doses. It is plausible that direct application of 7-OH-DPAT into the NAS shell fails to selectively stimulate sufficient D_3 DA receptors to induce inhibitory effects; rather, both behaviourally-inhibitory D_3 DA receptors and positively reinforcing D_2 DA receptors are concomitantly activated to produce little detectable change in reward-related behaviour.

Considering that many BSR reports should be interpreted with caution, as measures of response rates and rate-intensity analysis make it difficult to discriminate between reward and motor effects, the effects of 7-OH-DPAT on BSR have been inconsistent. Systemic studies have reported no effect of 7-OH-DPAT (Hatcher and Hagan 1998; Baldo et al. 1999a) or an increase or decrease in reward (Gilbert et al. 1995; Kling-Petersen et al. 1995b; Depoortere et al. 1996; Depoortere et al. 1999) and have often revealed biphasic dose-response functions. Given that these biphasic response patterns can also be seen with other aspects of conditioned reward (Sutton et al. 2001), it becomes increasingly evident that the effects of low doses of 7-OH-DPAT on reward are inhibitory, and that higher doses may be intrinsically rewarding. The shift from the inhibition of reward to facilitation of reward with higher doses of 7-OH-DPAT concurs with the theory described above that proposes the former may be primarily due to stimulation of

D_3 DA receptors, and the latter related to D_2 DA receptors. This theory may also be related to the time course of drug effects on behaviour. For example, it has been previously shown (Biondo 2002) that high doses of 7-OH-DPAT will induce CPP if it is administered 15 minutes prior to, but not immediately prior to, conditioning trials. The lack of effect of 7-OH-DPAT with zero delay may be related to the time-course of drug effects reflected by changes in locomotor activity. Khroyan et al. (1995) reported decreased locomotor activity 10 minutes after 7-OH-DPAT (5.0 mg kg^{-1}), but hyperactivity from 20-40 minutes. The initial inhibitory effect was attributed to stimulation of D_3 DA receptors, and latter stimulatory effects to D_2 DA receptor activation. In other words, in the context of place conditioning, a 15 minute delay may allow conditioning sessions to occur during a period of positively reinforcing D_2 DA receptor stimulation. Without the delay, conditioned reinforcing effects of D_2 DA receptor stimulation may be masked by (the potentially aversive) stimulation of inhibitory D_3 DA receptors. In conclusion, the contingency of DA receptor stimulation, of primarily D_2 DA receptors, may lead to the reward-related effects of high doses of 7-OH-DPAT.

The concept that stimulation of D_3 DA receptors may be inhibitory is also supported by recent systemic studies suggesting that D_3 DA receptor agonists may block reward-related behaviour in rodents. For example, 7-OH-DPAT has been shown to reduce amphetamine or cocaine-induced CPP (Khroyan et al. 1998; Khroyan et al. 1999) and decrease cocaine self-administration at doses that do not appear to serve as effective reinforcers (Caine and Koob 1993). In addition, the partial D_3 DA receptor agonist BP 897 may inhibit cocaine-seeking behaviour without having intrinsic or primary rewarding effects (Pilla et al. 1999) and the D_3 DA agonist quinelorane may block amphetamine-induced hyperactivity at doses that do not affect extracellular DA concentrations in the NAS (Thorn et al. 1997). These reports, in addition to the present set of experiments, suggest that D_3 DA receptors may be involved in the inhibition of reward processes, at doses that do not directly affect reinforcement when given alone. Based on independence from changes in DA release, it has been proposed that the D_3 DA receptors associated with these inhibitory effects may be located postsynaptically in the NAS (Waters et al. 1993; Svensson et al. 1994b,c; Mattingly et al. 1996; Depoortere et al. 1996; Zarandi 1998).

The inhibitory effects of low doses of 7-OH-DPAT may potentially be relevant to the pathophysiology of schizophrenia, since the positive symptoms of this disorder may be linked to excessive dopaminergic stimulation. Our knowledge of the contribution of D_3 DA receptors in this context would be greatly improved by the development of more selective D_3 DA receptor agonists that demonstrate lower affinities for D_2 DA receptors. In line with this hypothesis, several studies have suggested that partial D_3 DA receptor agonists may prove beneficial for the treatment of drug abuse or schizophrenia. Clinically, the $D_{2/3}$ DA receptor ligand amisulpride is also proving effective for management of schizophrenia and represents an improved pharmacotherapy that is well tolerated (McKeage and Plosker 2004). In the context of drug abuse, the D_3 DA receptor partial agonist BP 897 prevents cocaine-seeking in response to drug-associated cues (Pilla et al. 1999; Koob and Caine 1999). Other evidence suggests that D_3 DA receptors may be related to drug sensitization. A review and meta-analysis by Schwartz et al. (2000) suggested that excessive expression of D_3 DA receptors that has been observed in schizophrenia (Gurevich et al. 1997) may account for the behavioural sensitization to psychostimulants and, at least partially, for the psychotic symptoms. However, Richtand et al. (2001) have suggested that down-regulation of D_3 DA receptor function may contribute to behavioural sensitization induced by psychostimulants.

The behavioural effects of apomorphine are similar to those of 7-OH-DPAT; both compounds, at low doses, inhibit spontaneous locomotion and increase BSR rate-frequency thresholds. However, in contrast to 7-OH-DPAT, the behaviourally inhibitory effects of this dose of apomorphine have been attributed to the stimulation of D_2 DA autoreceptors (see page 24). That is, while apomorphine is a nonselective DA agonist, the dose used in this thesis has been widely shown to selectively stimulate D_2 DA receptors located presynaptically in the NAS (i.e., on terminals of DA neurons originating in the VTA) that would presumably alter DA release in the NAS. Like 7-OH-DPAT, apomorphine also demonstrates biphasic effects on behaviour across a range of doses; however, since only one dose has been used in this thesis, dose-related effects are not discussed here. Taken together, these behaviourally-similar but pharmacologically-different compounds were used to explore further DA-Glu interactions in relation to reward and reinforcement. The next section will discuss interactions of 7-OH-DPAT and apomorphine with Glu antagonists.

Interactions between Glu antagonists and DA agonists

The main findings for this section are as follows:

- Effects of systemic MK-801 and apomorphine on spontaneous locomotor activity did not interact (Experiment 1).
- Effects of systemic MK-801 and apomorphine on BSR rate-frequency thresholds did not interact (Experiment 5).
- There was significant interaction between effects of systemic MK-801 and 7-OH-DPAT on locomotor activity. Systemic 7-OH-DPAT (0.03 mg kg^{-1}) blocked locomotor stimulation induced by systemic MK-801 (0.03-0.13 mg kg^{-1}) (Experiment 2).
- There was significant interaction between intra-NAS effects of MK-801 and 7-OH-DPAT on locomotor activity. Intra-NAS shell 7-OH-DPAT (5.0 µg) blocked locomotor stimulation induced by intra-NAS MK-801 (0.66 µg) (Experiment 3).
- Effects of systemic administration of MK-801 and 7-OH-DPAT on BSR rate-frequency thresholds did not interact (Experiment 4).
- Effects of intra-NAS shell MK-801 (0.66 µg) and 7-OH-DPAT (5.0 µg) on BSR rate-frequency thresholds did not interact (Experiment 6).

- 7-OH-DPAT (5.0 mg kg^{-1}) induced conditioned place preference that was blocked by MK-801 (0.06 or 0.13 mg kg^{-1}) (Experiment 9).
- There was significant interaction between effects of intra-NAS NBQX and 7-OH-DPAT. Intra-NAS shell NBQX (0.5 μg) and 7-OH-DPAT (5.0 μg) combined increased BSR rate-frequency thresholds, indicating decreased reward (Experiment 7).

The effects of Glu receptor antagonism and DA receptor stimulation may not interact (i.e., may be additive) or the compounds may interact to produce either 1) the blockade of an effect; 2) the potentiation of an effect; or 3) a combined effect (i.e., synergy). Each of these relationships will be discussed in turn below. This is followed by a discussion of the potential mechanisms that may be associated with combined drug effects.

The effects of MK-801 and apomorphine were additive in studies of locomotor activity (Chapter 3) and VTA BSR (Chapter 4). To the best of our knowledge, there are currently no studies that have investigated the interaction between NMDA receptor blockade and selectively presynaptic D_2-like DA receptor stimulation in the context of reinforcement. As such, the present set of experiments is the first to demonstrate that the stimulatory effect of MK-801 and the inhibitory effect of low dose apomorphine on spontaneous locomotor activity or VTA BSR are additive; in other words, there was no interaction between NMDA receptor blockade and stimulation of (presumably presynaptic) D_2-like

DA receptors. Similarly, in studies of VTA BSR, effects of MK-801 and 7-OH-DPAT were also additive (Chapter 4). These additive effects contrast with a prior report that demonstrated decreases in rate-frequency thresholds induced by a combination of the nonselective DA agonist cocaine and MK-801 using lateral hypothalamic self-stimulation (Ranaldi et al. 1997). These differential effects are likely related to the selectivity (or lack thereof) of the DA agonist investigated.

In contrast to these additive or synergistic effects, a study of spontaneous locomotor activity has shown that 7-OH-DPAT may completely block hyperactivity induced by MK-801, and that the NAS shell is an important site for this effect (Chapter 3). Three reports have investigated the interaction between effects of MK-801 and DA agonists on locomotor activity. Dall'Olio et al. (1992) reported that repeated treatment with MK-801 for 21 days reduced hyperactivity induced by the D_2 DA agonist LY 171555. A second study explored acute effects of MK-801 and 7-OH-DPAT using habituated and non-habituated rats. In habituated rats (a similar methodology to the present study) Dall'Olio et al. (1997) reported that administration of MK-801 (0.06 mg kg^{-1}) or 7-OH-DPAT (0.005-0.08 mg kg^{-1}) did not induce any change in activity; MK-801 and 7-OH-DPAT combined were also ineffective. Using naïve rats with high baseline activity, Dall'Olio et al. (1997) reported that MK-801 (0.06 mg kg^{-1}) did not induce any change in activity, while 7-OH-DPAT (0.005-0.08 mg kg^{-1}) decreased activity. When combined, MK-801 reversed the decreases in activity induced by 7-OH-DPAT in naïve rats. These two studies suggest acute or chronic MK-801 may reverse effects of DA agonists; however, the results of Dall'Olio et al. (1997) are difficult to interpret in relation to the current study, as that study failed to demonstrate the locomotor suppressant effect of low dose systemic 7-OH-DPAT in habituated animals, and did not replicate the robust stimulant effect of MK-801.

Similar to the blockade effect of 7-OH-DPAT on MK-801 induced hyperactivity presented in this thesis, it has been recently shown that hyperactivity induced by MK-801 is also blocked by the D_3 DA receptor-selective partial agonist BP 897, the D_3 DA receptor-preferring antagonist nafadotride, or the atypical antipsychotic clozapine (Leriche et al. 2003). Though clozapine does have high affinity for the D_3 DA receptor, it is slightly less than that for the D_2 DA receptor (Sokoloff et al. 1990); nonetheless, Leriche et al. (2003) suggest that clozapine may inhibit MK-801 induced hyperactivity by blocking D_3 DA receptors. The similarity between effects of BP 897 and nafadotride are likely related to the observation that BP 897 may act in vivo as a D_3 DA receptor agonist or antagonist, depending on dopaminergic tone (Pilla et al. 1999). Taken together, these results add further evidence that the manipulation of D_3 DA receptors may be useful in the reversal of behavioural stimulation induced by NMDA receptor antagonists such as MK-801.

The experiments described above indicate that there are differential effects of 7-OH-DPAT and apomorphine on behavioural stimulation induced by NMDA receptor blockade, despite similar locomotor-suppressant effects of 7-OH-DPAT and apomorphine alone. These results have important implications for our understanding of pre- and postsynaptic DA-Glu interactions in motivation. Specifically, if both 7-OH-DPAT and apomorphine stimulated D_2-like DA autoreceptors to induce hypoactivity, then both of these DA agonists would be expected to have similar effects on behaviours induced by NMDA receptor blockade. That this similarity was not observed adds further evidence for the existence of behaviourally-inhibitory postsynaptic D_3 DA receptors, as described above. It is important to note that the reduction in activity following 7-OH-DPAT is not quantitatively different from that of apomorphine. For example, while 7-OH-DPAT alone reduced locomotor activity to approximately 62% of baseline, apomorphine decreased this measure to 65% of baseline. It is therefore likely that the *qualitatively* different effects of these ligands, rather than *quantitative* differences, contribute to differential interactions with MK-801. The qualitatively different effects of these ligands are likely related to the synaptic locations of action; while low doses of 7-OH-DPAT are likely activating D_3 DA receptors located postsynaptically, the dose of apomorphine used has been shown to selectively activates presynaptic D_2 DA receptors (see page 24).

These findings may extend further our knowledge of the role of D_3 DA receptors in psychosis and drug abuse. Specifically, our results suggest that stimulation of postsynaptic D_3 DA receptors, which are behaviourally-inhibitory, may be of therapeutic value in the treatment of drug abuse and psychosis related to schizophrenia. This potential is postulated since stimulation of D_3 DA receptors that are presumably postsynaptic effectively blocks the reward-related effects of

MK-801. This is relevant since MK-801 is an NMDA receptor antagonist that induces behaviours in laboratory animals that are similar to effects of psychotomimetic drugs, and other NMDA receptor antagonists such as PCP are chronically abused by humans. The neural basis for this DA-Glu interaction is likely related to the triadic synaptic arrangement of DA and Glu receptors on medium spiny neurons within the nucleus accumbens (see Sesack and Pickel 1992; Goldman-Rakic 1992). Specifically, the reward-related effects of NMDA receptor blockade may be due to decreased output of the NAS that is presumably GABAergic. In other words, the GABAergic medium spiny neurons of the NAS may be the postsynaptic neurons targeted by the DA projection from the VTA. NMDA receptor blockade may inhibit these medium spiny neurons of the NAS, since these neurons also receive Glu projections from the PFC (Sesack and Pickel 1992); NMDA receptor blockade would therefore attenuate excitatory input from the PFC, and disinhibit (i.e., activate) the VTA. This concept has been previously suggested for the reward-related effects of PCP in rats (Carlezon and Wise 1996).

The results of the BSR studies suggest that in electrical self-stimulation of the VTA, effects of blockade of NMDA receptors, particularly in the NAS, are independent of stimulation of both pre- and postsynaptic $D_{2/3}$ DA receptors. This finding may suggest that the compounds are not competing to affect the activity of neurons located postsynaptically in the NAS in the context of VTA self-stimulation. Specifically, if the compounds were acting to influence a common target, it would be expected that effects would not be additive; this was not the case. However, studies using nonselective DA receptor stimulation may indicate that this relationship does not apply to other DA receptor subtypes; recall that one prior report demonstrated rate-frequency threshold decreasing effects of a combination of the nonselective DA agonist cocaine and MK-801 on lateral hypothalamic self-stimulation (Ranaldi et al. 1997). Nonetheless, the non-additive effects, or interaction, between NMDA receptors and DA receptors observed in studies of locomotor activity (Chapter 3) taken together with additive effects in BSR (Chapters 4 and 5) may suggest that the neural substrates of DA and Glu that underlie spontaneous locomotor activity are dissociable from those implicated in circuitry of VTA BSR.

An important theoretical question is whether the interaction (or lack thereof) observed with NMDA receptor antagonists extends to non-NMDA receptor antagonists. The studies described in this thesis that have investigated NMDA–DA receptor interactions in the context of spontaneous exploration complements our prior studies using AMPA/kainate receptor antagonists. For example, although ineffective alone, AMPA/kainate receptor blockade by CNQX or NBQX has been shown to potentiate decreases in spontaneous locomotor activity induced by intra-NAS shell 7-OH-DPAT (Choi et al. 2000). In addition, intra-NAS shell or core CNQX has been shown to act interact with 7-OH-DPAT in VTA BSR to decrease reward (Choi 2000). In this thesis, intra-NAS shell administration of NBQX (Chapter 4) replicated this interaction by showing that intra-NAS NBQX and 7-OH-DPAT also combine to decrease VTA BSR.

As described above, concurrent stimulation of $D_{2/3}$ DA receptors by 7-OH-DPAT and NMDA receptor blockade resulted in additive effects, whereas AMPA/kainate receptor blockade did not. Taken together, these findings suggest that AMPA-DA receptor interactions are qualitatively different from NMDA-DA receptor interactions. Considering there are other studies showing differential effects of NMDA vs. AMPA/kainate receptor antagonists on the performance of some reinforcing tasks (Stephens and Cole 1996), clarification of DA-Glu interactions in the context of reinforcement and motivation are needed.

Differential AMPA-DA and NMDA-DA interactions have important implications for our understanding of the in vivo circuitry of the mesolimbic system. The theory described at the beginning of this discussion suggested that activation of AMPA/kainate receptors might provide the membrane depolarization necessary for the removal of Mg^{2+} blockade from the NMDA receptor. If so, blockade of AMPA/kainate receptors should produce similar effects to those observed with NMDA receptor blockade alone. However, this theory is not supported by the present set of experiments. Differential effects of AMPA/kainate vs. NMDA receptor blockade in BSR are not consistent with a role for AMPA-induced depolarization as an enabling mechanism for NMDA receptor function. As such, we suggest that depolarization mediated by non-AMPA receptors may enable NMDA receptor function and related reward-altering behavioural effects of drugs such as MK-801. Other classes of Glu receptors, such as metabotropic Glu receptors, may mediate this depolarization.

Though metabotropic Glu receptors are not defined as ion channels, activation of related G-proteins could, either directly or indirectly, affect membrane ion channels to induce the membrane depolarization needed to enable NMDA receptor activation. Consistent with this concept, an emerging role for metabotropic Glu receptors in the context of reward is discussed below (see page 241).

The mechanism by which AMPA/kainate receptor antagonism acts in combination with 7-OH-DPAT to decrease reward is unclear. Evidence suggests that intra-VTA CNQX does not affect DA in the NAS (Karreman et al. 1996; Mathé et al. 1998); however it has been shown that administration of intra-NAS $D_{2/3}$ DA agonists may reduce extracellular Glu concentrations in the NAS (Kalivas and Duffy 1997). This effect was interpreted in terms of stimulation of DA receptors located on Glu-containing terminals of cortical projections to the NAS. Therefore, it is possible that 7-OH-DPAT induced a decrease in Glu transmission in the NAS, and this effect coupled with increased blockade of AMPA/kainate receptors to decrease Glu transmission further. Further investigation of this hypothesis has important implications for understanding the mechanisms of effects of AMPA/kainate receptor blockade on the actions of indirect DA agonists, such as cocaine and amphetamine, on indices of motivated behaviour (Kaddis et al. 1993; Burns et al. 1994; Wan and Swerdlow 1996).

It must also be noted that the study of place conditioning (Chapter 6) has extended our understanding of NMDA-DA receptor interactions to conditioned reward. However, an important difference should be noted: the dose of 7-OH-DPAT used to induce CPP is a "high" dose and likely stimulates both pre- and postsynaptic $D_{2/3}$ DA receptors (see page 220-221); this stands in stark contrast to the studies described above that used much lower doses of 7-OH-DPAT to stimulate primarily postsynaptic D_3 DA receptors.

CPP induced by high doses of 7-OH-DPAT was blocked by systemic administration of MK-801. This finding is consistent with a prior study from this laboratory that showed 7-OH-DPAT induced CPP is blocked by the intra-NAS shell administration of the AMPA/kainate receptor antagonist NBQX (Biondo 2002). These results are also consistent with effects of less selective DA agonists. For example, intra-NAS DNQX suppressed or blocked CPP induced by amphetamine (Layer et al. 1993b) or cocaine (Kaddis et al. 1995). Therefore, stimulation of Glu receptors in the NAS may be necessary for the acquisition of conditioned reinforcement to indirect DA agonists and selective $D_{2/3}$ DA receptor agonists (this was discussed in Chapter 6).

Nucleus accumbens

The NAS is a major component of the ventral striatum (Heimer and Wilson 1975) that may be divided into core and shell subregions (see Chapter 1). Deutch et al. (1993) proposed that the NAS shell may be more involved in motivation and the core more related to motor function. Therefore, based upon the functional heterogeneity of the NAS and previous results from this laboratory

(Choi 2000; Biondo 2002), microinjections were selectively limited to the NAS shell subregion. Of particular importance to the present thesis, recent studies have suggested that the NAS shell subregion may be comprised of at least three compartments based upon immunostaining and tract-tracing studies (Groenewegen et al. 1999; Zahm 1999). Although the precision of microinjection procedures is limited by the diameter of the infusion tip, further study of the subregions and subcompartments of the NAS should continue. Such detailed understanding of the accumbens neuroanatomy may yield valuable information that may be exploited when more precise methods and materials for microinjections are developed using nanotechnology in the future.

The NAS is an important site of DA-Glu interactions in relation to reward and reinforcement, as it is involved in motivation and goal-directed behaviours (Mogenson et al. 1980). There is currently a paucity of data to describe the role of Glu in the NAS on reward. Heidbreder et al. (1992) demonstrated that administration of Glu into the caudal NAS decreased rates of LH BSR. Kelley and Throne (1992) showed that intra-NAS AP5 can attenuate amphetamine potentiation of responding for conditioned reward. This result was confirmed and expanded upon when Burns et al. (1994) systematically investigated the effects of a range of Glu receptor-related compounds on this task. In that study, both agonists such as NMDA and AMPA, and antagonists such as AP5 and CNQX, blocked the potentiation of conditioned reward by amphetamine. Maldonado-Irizarry et al. (1995) showed that blockade of AMPA/kainate receptors induced feeding following microinjection into the NAS shell, but not core. Subsequently, Carlezon and Wise (1996) showed that intra-NAS shell NMDA receptor antagonists (PCP, MK-801 and CPP) decrease rate-frequency thresholds of LH BSR. The experiments described in this thesis have built upon these studies to make a meaningful contribution to our knowledge regarding the role of Glu in the reward pathways of the brain.

Limitations

The specificity and selectivity of pharmacological compounds is of great importance to the accurate interpretation of experimental results. Therefore, it is noteworthy that 7-OH-DPAT and noncompetitive NMDA antagonists such as MK-801 may interact with σ binding sites (Rothman et al. 1992; Schoemaker 1993; Maurice et al. 1994a,b; Debonnel and de Montigny 1996; Ault and Werling 1999). In addition, there is also evidence to suggest that σ receptor ligands may alter Glu activity in the mesolimbic system (Gronier and Debonnel 1999). As such, the contribution of σ receptors to DA-Glu interactions should be noted, and may be of interest in the interpretation of future studies. In addition, although MK-801 is considered a noncompetitive NMDA receptor antagonist, there is some evidence to suggest a lack of specificity. For example, MK-801 has been shown to attenuate, but not block, behavioural impairments induced by NMDA receptor agonists (e.g., Zajaczkowski et al. 1997). In addition, the hyperactivity induced by MK-801 was antagonized by intra-VTA CNQX, suggesting that

AMPA/kainate receptor activation may also underlie behavioural effects of MK-801 (Mathé et al. 1998). It is also noteworthy that while apomorphine is a nonselective DA agonist, the dose employed in this thesis has been shown to induce behavioural effects via stimulation of D_2-like, but not D_1-like, DA receptors (see page 24). Therefore, for interpretation of the experiments described above, effects of this particular dose of apomorphine may be considered D_2-like DA receptor-related effects.

Related to drug specificity, the selectivity of 7-OH-DPAT has been questioned in one study using D_3 DA receptor knockout mice. In that study (Boulay et al. 1999), which used higher doses of 7-OH-DPAT (0.3-3 mg kg^{-1}) than used in the present study, 7-OH-DPAT induced changes in locomotor activity in mice lacking D_3 DA receptors. This finding is consistent with the proposal that higher doses of 7-OH-DPAT induce behavioural effects via stimulation of D_2 rather than D_3 DA receptors. It is noteworthy that the contribution of compensatory mechanisms that may have adapted for the absence of D_3 DA receptors is unclear.

Although in the present set of experiments systemic drug effects paralleled those found with intra-NAS shell microinjections, the process of direct drug application into the brain has some limitations (see Carvey et al. 1994; McBride et al. 1999). The primary limitation of this method is related to drug diffusion based on the lipophilicity of the microinjected compounds. Diffusion in tissue generally occurs in a spherical gradient; because most anatomical structures are not spherical, there is generally nonspecific drug exposure to nearby structures, or insufficient exposure to the whole of the site of interest. It is noteworthy that the present set of experiments used a minimal microinjection volume to diminish diffusion outside of the NAS shell; in addition, the location of infusion sites has been verified histologically. This is important as repeated drug infusions into the brain may cause damage to tissue. This risk was minimized in the present set of experiments by limiting the number of microinjections to four per animal with three drug-free days between infusions to allow for drug washout.

There are also limitations to the BSR paradigm. BSR is a relatively uncommon but highly valued method for assessing reward-related drug effects. However many reports of lever-pressing for BSR need to be interpreted with caution, as some methods may not differentiate changes in reward from nonspecific performance-related effects. The present set of experiments employed rate-frequency threshold measures that are considered somewhat "rate-independent" (see Greenshaw and Wishart 1987; Liebman 1989; Wise and Rompré 1989). Another limitation of BSR is analogous to the above discussion of drug diffusion. Ranck (1975) described how electrical stimulation may extend into nearby brain regions away from the stimulating electrode in a manner proportional to the electrical parameter of current; this was elaborated upon by Fouriezos and Wise (1984). Although BSR may be maintained by numerous brain regions, the present set of experiments has employed electrical stimulation of the VTA; this area contains mainly DA-containing neurons that innervate the NAS.

When combined with relatively low stimulation currents (typically <200 μA), electrical stimulation of the VTA is presumably relatively specific to allow for interpretation of results in relation to selective activation of the mesolimbic DA system.

Another limitation of studies of behavioural pharmacology that investigate DA-Glu interactions is related to the age and strain of the animals used. Recently, Segovia and Mora (2005) have demonstrated that there are differential effects of Glu agonists on DA release in the NAS; rats aged 10-14 months show significantly lower increases in DA relative to rats aged 2-4 months. In addition, it has been recently shown that Wistar and Sprague-Dawley rats demonstrate differences in DA receptor binding; specifically, Wistar rats show higher D_1, D_2, and DA transporter binding than Sprague-Dawley rats, while there were no differences in D_3 DA receptor binding between the two strains (Zamudio et al. 2005). Together, these recent studies suggest that the age and strain of animals should be noted and carefully considered when interpreting results of studies that have investigated DA-Glu interactions.

Conclusions and future research

The present set of experiments can be summarized as follows. NMDA receptor blockade by MK-801 induced robust hyperactivity and facilitated ventral tegmental brain stimulation reward; the NAS shell subregion was a sufficient site for these effects. Dopamine D_2-like receptor stimulation by low dose apomorphine or 7-OH-DPAT have opposite effects than MK-801; both induce hypoactivity and decrease brain stimulation reward. Effects of NMDA receptor blockade and apomorphine were additive (i.e., did not interact) in both studies of locomotor activity and brain stimulation reward, suggesting MK-801 effects are independent of DA release in the NAS. In addition, the effects of NMDA receptor blockade and 7-OH-DPAT were also additive (i.e., did not interact) in brain stimulation reward, whether the drugs were given systemically or directly into the NAS shell, suggesting MK-801 effects are independent of postsynaptic DA receptor stimulation. The two significant interactions found in the present set of experiments were: 1) 7-OH-DPAT completely blocked the locomotor stimulatory effect of MK-801, both systemically and in the NAS shell subregion; and 2) administration of NBQX and 7-OH-DPAT into the NAS shell, that were both ineffective when given alone, decreased brain stimulation reward when given in combination.

The balance of DA receptor subtype stimulation may play an important role in functional DA-Glu interactions. Future experiments could therefore explore effects of other Glu receptor-related ligands alone and in combination with nonselective DA agonists or selective D_1-like agonists. The interaction, or lack thereof, between D_1-like DA receptor stimulation and NMDA or AMPA receptor antagonism in brain stimulation reward may be a valuable area of future research. Despite the lack of interaction between D_1-like agonists and Glu ligands in a study of locomotor activity (Choi et al. 2000), GluR1 expression is increased in the NAS following administration of the D_1 DA receptor agonist SKF 81297 (Chao et al. 2002).

An emerging area of research related to DA-Glu interactions in the NAS is the role of adenosine. Adenosine has opposite modulatory roles on extracellular concentrations of DA and Glu in the striatum. Harvey and Lacey (1997) suggested that stimulation of D_1 DA receptors inhibits Glu in the NAS via adenosine release that is mediated by NMDA receptor activity. Studies of adenosine receptor ligands have also indicated a role for adenosine in primary reward (Baldo et al. 1999b) or conditioned reinforcement to amphetamine (Poleszak and Malec 2003). Quarta et al. (2004) recently reported that modulation of DA release in the NAS shell by adenosine receptors is dependent upon NMDA receptor stimulation. These studies underlie the need to fully explore interactions between both D_1-like and D_2-like DA receptors with both NMDA and non-NMDA receptors in the context of reinforcement.

In addition, there is an emerging role for cannabinoid (CB) receptors in reward and reinforcement. For example, Houchi et al. (2005) have recently shown that CB_1 knockout mice demonstrate increased $D_{2/3}$ DA receptor binding sites, and show altered locomotor responses to the $D_{2/3}$ DA receptor agonist quinpirole. In addition, Soria et al. (2005) recently demonstrated that CB_1 knockout mice fail to self-administer cocaine. The contribution of CB receptor activity to DA-Glu interactions may be relevant to the study of psychiatric disorders, since the endogenous CB system is associated with several neurological and psychiatric conditions, including drug abuse (Van der Stelt and Di Marzo 2003).

There is also increasing evidence for a role of drug-induced synaptic plasticity in reward. Jones and Bonci (2005) have recently suggested that NMDA-dependent long term potentiation (LTP) in the VTA may contribute to the acquisition of instrumental or Pavlovian learning in response to the administration of addictive drugs. Further investigation and characterization of the role of Glu in reward-related learning will aid in our understanding of the neural circuitry of conditioned drug reward, which is relevant for the study of, and development of potential treatments for, drug abuse.

As described in the present set of experiments, DA and Glu receptor-related compounds may or may not interact depending on the specific compounds and behavioural measures. As such, several experiments may help to clarify DA-Glu interactions in the context of motivation. Future work could explore the effects of competitive NMDA receptor antagonists, or explore the role of the glycine site of the NMDA receptor in reward. There are also few data available that describe effects of Glu uptake inhibitors in this context. Lastly, given that recent data indicate that metabotropic receptors (specifically mGluR5) may be associated with changes in reinforcement, effects of selective mGluR5 ligands should be assessed. Also, the contribution of other mGluRs should be evaluated; Morishima et al. (2005) recently demonstrated that place preferences to cocaine are enhanced in mGluR2 knockout mice, which also show altered extracellular

DA and Glu responses to cocaine. The contribution of specific brain regions such as the VTA, NAS, and PFC to these effects could also be determined by studies using central drug administration.

Such systematic investigations will help to fully characterize functional DA-Glu interactions in the mesolimbic system. As NMDA receptors, AMPA/kainate receptors, and D_2-like DA receptors have all been implicated in the neural circuitry underlying some psychiatric disorders of motivation, behavioural interactions of $D_{2/3}$ DA receptors and the excitatory amino acid system within the midbrain may be a potential target for therapeutic drug development. In addition, further investigation of DA-Glu interactions (and associated effects on motivated behaviour) are important for understanding the pathophysiology of, and therefore the development of novel treatments for, drug abuse and schizophrenia.

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Appendix A. Drug preparation

All doses of drugs used in this thesis are expressed as free base doses. Below are the conversion factors and any special notes for each compound.

Compound	**Formula**	**True Molecular weight**	**Actual Formula weight**	**Conversion factor**
MK-801 maleate	$C_{16}H_{15}N{\cdot}C_4H_4O_4$	221.30	337.37	0.656
NBQX disodium	$C_{12}H_8N_4O_6S{\cdot}Na_2$	336.28	380.24	0.884
7-OH-DPAT hydrobromide	$C_{16}H_{25}NO{\cdot}HBr$	247.38	328.29	0.754
Apomorphine hydrochloride hemihydrate	$C_{17}H_{17}NO_2{\cdot}HCl{\cdot}\frac{1}{2}H_20$	267.33	312.79	0.855

- MK-801 and NBQX solids may be kept at room temperature.
- 7-OH-DPAT and apomorphine solids are to be stored in a container with desiccant and kept in the refrigerator.
- All solutions of these compounds should be made fresh daily.
- 7-OH-DPAT is light sensitive, and so it should always be wrapped in aluminum foil.
- Apomorphine is light and temperature-sensitive, and so it should always be wrapped in aluminum foil and kept on ice.

Appendix B. Cerebrospinal fluid preparation

Three solutions (A, B, and C) are required to make the final CSF preparation (D).

Stock Solution A: Alkaline-Salt Solution

Dissolve the following reagents in 1 liter of distilled water. Keep the solution tightly sealed in a screw-top bottle.

Sodium chloride	236 g
Sodium carbonate	92 g
Potassium chloride	12 g
Disodium hydrogen phosphate dodecahydrate	8 g

Stock Solution B: Acid-Salt Solution

Dissolve the following reagents in concentrated hydrochloric acid. If necessary, gentle warming may be used to aid in dissolution of the magnesium salt; however, keep it below 55°C. Keep the solution tightly sealed in a screw-top bottle.

CAUTION: this solution is corrosive.

Calcium chloride	24 g
Magnesium chloride hexahydrate	12 g
Concentrated hydrochloric acid	300 mL

Appendix B. Cerebrospinal fluid preparation (continued)

Solution C: Glucose Solution (not a stock solution – make it fresh each day)

Dextrose anhydrous	0.4 g
Distilled water	25 mL

Solution D: Final CSF solution for use in vivo

1. In a 100 mL volumetric flask, dilute 2.5 mL of stock solution A to 100 mL with distilled water.
2. In a 250 mL flask, combine the entire solution from step 1 with 5.0 mL of solution C.
3. Using a pH meter, carefully adjust the pH to 7.1-7.2 by adding a small quantity of solution B. (You will need approximately 0.200-0.300 mL).
4. Suction filter the solution from step 3 into a 250 mL suction flask through a 0.2 μm nylon membrane filter.
5. De-gas using the separator filter on same apparatus for at least 10 minutes.
6. Aliquot the solution into 1.5 mL microfuge tubes for in vivo injection within 5-6 hours (the pH will drift over time).

Note: Prepare solution D immediately prior to use in vivo. Do not warm it above 55°C and do not expose it to air any longer than necessary.

Appendix C. Standard operating procedures

In vivo implantation of central electrodes and bilateral cannulae in rats

Introduction

Animals that undergo the invasive procedure described below are intended to fully recover from the operation and perform rigorous behavioural tests. Therefore the procedure described in this appendix requires that the operator follow strict aseptic technique to ensure the animal does not suffer infection. It is not possible to maintain a completely sterile working environment, but items that have not been disinfected should not enter the "surgical field." The operator is required to wear a facemask, back-fastening gown with tight arm cuffs, and latex gloves. During the entire procedure, gloved hands should be disinfected frequently using antiseptic hand cleanser containing 2% chlorhexidine.

The following list serves as a checklist of supplies – each of these items should be available and ready to use before beginning.

Appendix C. Standard operating procedures (continued)

Stereotaxic frame

Cauterizer + 2 AA batteries

Bead sterilizer

Dremel™ (with pen attachment)

Dental burr tip

Drill bit + pin vise

Flathead jeweler's screwdriver

Cordless clippers

Heat lamp

Narrow-wire cage lids

1 package paper towels

1 incontinence pad

1 box cotton tipped applicators

20-30 Kimwipe™ tissues

1 large weigh boat

8-10 small weigh boats

50 mL saline

50 mL 70% anhydrous ethanol

5 mL 100% anhydrous ethanol

4 jeweler's screws

2 beakers (100 mL, 250 mL)

1 scalpel with #10 blade

1 spatula

1 forceps (with teeth)

1 forceps (fine)

4 hemostats

1 micro spatula

1 probe

Wound clip applicator + 3 clips

Stainless steel instrument dish

Halothane

Xylocaine

Germex

Orthodontic resin and cement

60-second Fluoride Treatment

Instrument preparation

All instruments (after being cleaned of debris) including the drill bit, dental burr, and jeweler's screwdriver, should be immersed into Germex solution within the stainless steel instrument dish for at least 10 minutes, but preferably overnight. Afterward, transfer these items into the 250 mL beaker filled with Germex solution and keep them in this solution throughout the procedure. Devices to be implanted (e.g., guide and dummy cannulae, electrode, ground electrode) as well as the jeweler's screws and wound clips should be placed into 70% anhydrous ethanol within the large weigh boat and kept in this solution throughout the procedure. Note that in order to be considered cleaned by the ethanol, the item must be in solution for at least 10 minutes.

Components

Depending on the type of surgery, the following components will be required: guide cannulae, dummy cannulae (custom cut to extend 1 mm beyond the tip of each guide cannula and then polished smooth), an electrode (custom cut to 18 mm), a ground electrode (handmade using a socket contact soldered to 1 cm of silver wire soldered to a jeweler's screw), and an electrode pedestal with pedestal cap.

Stereotaxic alignment

All components to be implanted using the stereotaxic apparatus are first aligned (straightened) and then zeroed. The cannulae and electrode are aligned horizontally and vertically using the ear bars as a guide. Once aligned, the stereotaxic arms are set at desired angles and the components zeroed according to interaural zero (IAZ). To set IAZ, the ear bars are set to 0.5 mm on each side. The 1 mm gap in between is considered IAZ. To zero each component, the very tip is centered in three dimensions to the exact center of this gap. Once centered, the stereotaxic coordinates for each component are recorded and used to calculate target coordinates. The stereotaxic arms are then removed from the stereotaxic and placed in a safe place where they will not accidentally be bumped and misaligned.

Animal preparation

In an area separate from the surgical setup, the animal weighing between 300-350 grams is anesthetized using inhalation Halothane (5%). Once under anesthesia, the concentration of Halothane is reduced to a level required to maintain anesthesia (between 1-2%). The head is shaved and the animal transferred to the stereotaxic frame. Once the animal is secured and centered in the stereotaxic apparatus, the "surgical field" of the head is cleaned using 4-5 cotton-tipped applicators soaked in Germex solution. The head is then cleaned using 4-5 cotton-tipped applicators soaked in 70% ethanol. It is most effective to begin in the center of the surgical field and make consecutively larger concentric circles to the perimeter.

Implantation

Make an incision approximately 2 cm in length beginning just posterior of the eyes and proceeding rostral. Using the spatula, scrape away all connective tissue from the bone surface. Dry the area with cotton-tipped applicators. Using the forceps with teeth to grasp the perineum, clamp the tissue with hemostats and pull away in the direction of the four corners. Using the cauterizer, stop the bleeding from any areas on the skull that are seeping. Clean the bone surface again using cotton-tipped applicators dipped in saline. Do this 3-4 times until the applicators, when wiped on the bone, come away clean. Apply 60-second Fluoride Treatment. When complete, clean the bone with cotton-tipped applicators dipped in saline. Bring in the zeroed cannulae or electrode to estimate the position of entry through the skull. Remember the approximate position and return the cannulae and electrode to a safe place to avoid bumping.

Using the handheld drill bit, drill four holes in the skull forming a square, ensuring that the placement of screws does not overlap with the eventual positioning of cannulae or electrodes (optional: place a 5th hole on the midline directly posterior to lambda). Into these holes, place a jeweler's screw (for bilateral cannulae implants) plus one ground electrode (for electrode implants). The ground electrode is placed into the hole in the southwest quadrant. If any bleeding occurs during this process, clean the bone again as described above using cotton tipped applicators dipped in saline. Once all bleeding has stopped, apply a drop of 70% ethanol to the skull, followed by 1-2 drops of 100% ethanol. This will dry up the bone surface completely, when allowed to air dry for approximately 2 minutes. When dry, use the blunt probe to etch grooves into the area of the skull upon which you plan to apply dental acrylic. Grooves should be placed at various angles (forming a hatched pattern) and should be spaced approximately 1 mm apart.

Prepare dental acrylic by mixing the cement powder with the resin liquid. The consistency for the first layer that contacts the bone should be quite fluid to allow the compound to seep into the natural contours of the bone and as well into the etched grooves. Apply the base layer ensuring to encompass the screws to form an oval-shaped foundation. The foundation should be as wide as possible without compromising the cement edge. It is essential that the bone is absolutely dry or the dental acrylic will not bond properly. Subsequent layers of cement will only build upon this foundation, so ensure that the foundation is wide enough to support the remainder of the implant to be built. Once the base layer has been applied, allow it to set thoroughly – wait a full 10 minutes or more. While waiting, position the components to implant and mark the locations of holes to be drilled onto the cement surface. When the cement is completely dry, use the Dremel™ and dental burr tip to drill the holes for the implant components through the dental acrylic and skull surface, being careful not to damage dura. If any bleeding occurs, use cotton-tipped applicators to keep the area clean and dry.

If bilateral cannulae are being implanted, the holes may be drilled and cannulae put into place simultaneously. If the implant will have an electrode in addition to bilateral cannulae, it is necessary that the hole for the electrode be drilled and the electrode held into place with cement before the bilateral cannulae (otherwise the cannulae will interfere with placement of the electrode carrier of the stereotaxic).

Once the implant components have been stereotaxically lowered into position, ensure the holes no longer bleed and that all surfaces are dry. Prepare another batch of dental acrylic, this time adding more powder relative to fluid to create a less-fluid mix. Thicker cement is desired as it will harden faster and reduce the time needed to complete the surgery. Use this mix to fill in the holes surrounding the implanted components.

For surgery to implant cannulae, do this repeatedly until the cement encases the threads at the end of the cannulae. The rest of the implant may then be shaped by applying as many layers as needed to produce an implant with smooth edges all around. When the implant is completely set (wait at least 10 minutes, depending on the cement thickness used), remove the stereotaxic arms very carefully, ensuring very little upward pressure is being applied to the implant.

For surgery to implant an electrode, place only one layer of cement and let it set for at least 10 minutes. When set, remove the stereotaxic electrode carrier and bend the electrode rostral and apply another layer of cement to encase the elbow joint (this is necessary to ensure that the electrode does not move later on). When the cement applied to the elbow is completely set, the sockets of both electrodes may be manually manipulated to be situated into the electrode pedestal. The closer to the skull the pedestal rests, the better – an implant with a lower profile is less likely to be damaged after the animal has recovered. Once the pedestal is in place, mix dental cement to create a less-fluid mixture. Apply repeated layers of this mixture around the pedestal. Be careful though – if the cement is too fluid, it will fill the holes of the pedestal due to capillary action and the pedestal will not connect to the brain stimulation reward apparatus. The rest of the implant may then be shaped by applying as many layers as needed to produce an implant with smooth edges all around. When the implant is completely set, close the wound.

Closing the wound

Once the implant has hardened, the wound is briefly cleaned using saline-soaked cotton tipped applicators. Then the incision is closed using wound clips. Keep in mind that the surrounding tissue has swollen during surgery and will reduce in size during recovery – so do not apply the wound clips too tightly around the implant. However the wound clips should sit close enough to the implant so as not to create an open pocket for debris. Lastly, Xylocaine should be applied around the wound using cotton-tipped applicators.

Post-operative care

Once the anesthetic is off, the animal is removed from the stereotaxic apparatus. The animal is then placed on its side into a clean cage with paper towels covering the bedding, located under a heat lamp. Ensure the animal's tongue is exposed and resting against the cheek to ensure adequate respiration.

Check to ensure the heat lamp is not too close or too hot by placing your hand beneath. Flip the animal to its opposite side every few minutes and provide tactile stimulation to enhance its rate of recovery from anesthesia. When mobile, the heat lamp may be removed and a cage lid prepared. Ensure the cage lid has narrow spaces between the wires.

Place food into a stainless steel food dish and place the dish onto the cage floor. For more clearance within the cage, attach the cage lid upside down using twist ties to keep the lid in place. Prop up the water bottle on top of the cage lid. Note that the food dishes must be cleaned in advance by scrubbing them with a test-tube wire brush, followed by disinfection by immersion into Germex solution.

Recovery

Animals should be monitored each day following implantation to ensure good health. Implants should be inspected regularly for signs of infection or bleeding. Approximately 7 days following implantation, the wound should be healed completely and the animal should have regained its full pre-operative weight. Throughout the experiment, the animal should be monitored for implant integrity and overall well-being. If at any time an animal seems to experience distress due to dislocation of the implant, that animal should be terminated immediately.

Microinjection procedure

The microinjection procedure is described in the *Materials and methods* section of the thesis. This paragraph is intended to elaborate on that procedure.

Each microinjection requires the animal be held still for several minutes. This is very easy to do, and is not stressful for the animal, if the animal has had plenty of prior handling to get accustomed to the experimenter. Prior to preparing the animal for injection, ensure your tubing and injector cannulae are ready. No bubbles should be evident (other than the bubble used to indicate fluid movement in the tubing). Settings on the syringe pump must be correct (and may need to be calculated depending on syringe size). The clock timer is also set in advance (timer 1 = 2 min 30 s; timer 2 = 1 min).

The animal is held lightly under a towel with only the head exposed. Gently, the dummy cannulae are removed and placed into 70% ethanol, keeping track of which one came from the left and right side. The injector cannulae are fully inserted into the guide cannulae. The pump is turned on and clock timer 1 is started. The injection takes exactly 2 minutes 30 seconds at which time the pump is turned off and the injection cannulae left in place for another 1 minute (timer 2). During this time, the dummy cannulae are removed from the 70% ethanol and dried. The injector cannulae are then gently removed and dummy cannulae replaced carefully. The animal is now ready for a behavioural testing procedure.

Appendix D. Histology protocols

The histology protocol is described in the *Materials and methods* section of the thesis. This appendix is intended to elaborate on that procedure.

Phosphate buffered saline (PBS – 10 mM)

1. Add 800 mL double distilled water to a 1000 mL beaker.
2. Add, while mixing with a magnetic stirring bar:

Potassium chloride	0.2 g
Potassium phosphate monobasic	0.2 g
Sodium chloride	8.0 g
Sodium phosphate dibasic anhydrous	1.42 g

3. Keep stirring until dissolved.
4. Adjust the pH to 7.2-7.4 using hydrochloric acid or sodium hydroxide.
5. Transfer the solution to 1000 mL volumetric flask and top up the volume to 1000 mL with distilled water.
6. Transfer the solution to a glass storage container and store it in the refrigerator.

30% sucrose in PBS:

1. Add 300 mL PBS to a 500 mL flask with a magnetic stirring bar.
2. Add, with stirring, 90 g sucrose.
3. Stir until dissolved (10-15 min).
4. Transfer the solution to a glass storage container and store in the refrigerator.

Using the cryostat

One hour beforehand, remove a frozen brain from the –80°C freezer and place it inside the cryostat. Take the brain out of the vial and place it on filter paper on top of the mounting plate. This allows the brain to warm up from –80°C to –20°C.

Have some microscope slides labelled in preparation for mounting of tissue. Always use pencil and keep the label simple. Keep a master record sheet to match the details of each slide to its unique identifier. Consider using sets of 4 slides labelled sequentially to avoid putting serial slices on the same slide. This way if you lose or break a slide, the neighboring slices are not lost as well.

Keep brushes, tools, and slides inside the cryostat so that they do not warm up to room temperature.

Appendix D. Histology protocols (continued)

Tissue preparation

Remove the mounting block from the cryostat (the mounting block is the steel quarter-sized pedestal with circled texture). Let it warm up to room temperature, and then apply a coating of Jung Tissue Freezing Medium to the mounting block and place it inside the cryostat. Wait several minutes for it to cool and harden (it will turn solid white).

The following steps are best done inside the cryostat so that the brain does not warm up.

While waiting for the brain to warm up to –20°C, you may want to "block" the brain. This means to chop off a section of the brain with a scalpel or razor blade so that you may begin to section more posterior and closer the region of interest, instead of sectioning the entire brain. Be careful not to block too near (or beyond) the area of interest.

Remove the mounting block from the cryostat and add a few more drops of Jung Tissue Freezing Medium. Immediately take the frozen brain and place it on the drops of liquid medium, making sure that if you want coronal sections the brain should be "stood up" with the most posterior region acting as a "foot" and placed into the medium. Place the mounting block and brain into the cryostat for a few minutes to cool and harden.

Using a gloved finger, apply additional medium as needed around the base of the brain. As some mechanical forces are generated during sectioning, it is essential that the mounted brain is stable.

There are several tips that you may find useful for using the cryostat.

Before beginning to section a brain, the glass roll plate should be properly aligned to ensure that each time a slice is made, the tissue section falls beneath the roll plate. To align the roll plate, it should rest almost exactly on the edge of the blade, but rest a hair closer to the user.

Be familiar with the landmarks of interest for the brain region(s) you wish to examine. Check out the brain atlas of Paxinos and Watson (1986) and prepare the measurements of slice width and number of slices needed according to the location of the region of interest.

Establish a "brain library" or other system to keep your collection of sectioned brains organized. If you wish to examine more than one brain area, be prepared before slicing to know exactly how many slices will be produced from the region, and how many slides will need to be labelled.

Temperature is often key to obtaining good slices. If the brain is flaking and cracking easily, the temperature is likely too cold. If you find that the blade is sticking on its way through the brain – almost denting the brain when it first makes contact – then likely the temperature is too warm. After slices have been expelled and come to rest under the glass roll plate, leave the mounting block below the blade. Lift the roll plate to expose the tissue slice and then mount it.

Mounting can be done various ways – you can bring the appropriate microscope slide from outside (where it is warm) into the cryostat. Slowly bring the charged surface into contact with the edge of the tissue slice and the remainder of the slice should attach itself properly. Alternatively, you can transfer the slice onto microscope slides kept inside the cryostat (where it is cold) and set tissue slices in place using a brush. This way, you can move the slice around somewhat and control the warming of the slide using your finger on the reverse side of the slide, which is less likely to produce bubbles.

Poor slices may result if the tissue sticks to the blade or roll plate. If this occurs, clean the edge of the roll plate and the edge of the blade with an ethanol-moist cotton-tipped applicator. Poor slices may also occur if tissue or medium are hanging loosely from the edges of the mounted tissue. The top and bottom edges should be parallel to the blade to ensure even slices – trim with a razor blade as needed. Also try smoothing the edges with a brush or by rubbing your thumb or finger along the rough edge. Be careful – your hands are very warm relative to the tissue and you don't want the tissue to warm up too much. If there is a large temperature difference between the tissue and the mounting stage, the slice will curl after being expelled under the roll plate. Close the lid and let the cryostat components cool for at least 15 minutes before sectioning again.

There are a number of options for staining slides. For the histology presented in this thesis, Nissl staining was used. The remainder of this appendix will therefore describe how to prepare the Nissl stain and apply it to your slides.

Appendix D. Histology protocols (continued)

Stock solution of cresyl violet (Nissl) stain:

1. Solution A: Sodium acetate (1M)

Sodium acetate	68 g
Distilled water	500 mL

2. Solution B: Acetic acid (1M)

Glacial acetic acid	60 mL
Distilled water	940 mL

3. 0.5% cresyl violet (pH~3.9):

Cresyl violet	5 g
Distilled water	600 mL
Solution A	60 mL
Solution B	340 mL

4. Cover or seal the solution, and mix for 3-7 days on magnetic stirrer.
5. Filter the solution using a Whatman #4 filter and a Buchner funnel. This will take a very very long time (on the order of days), so be patient.

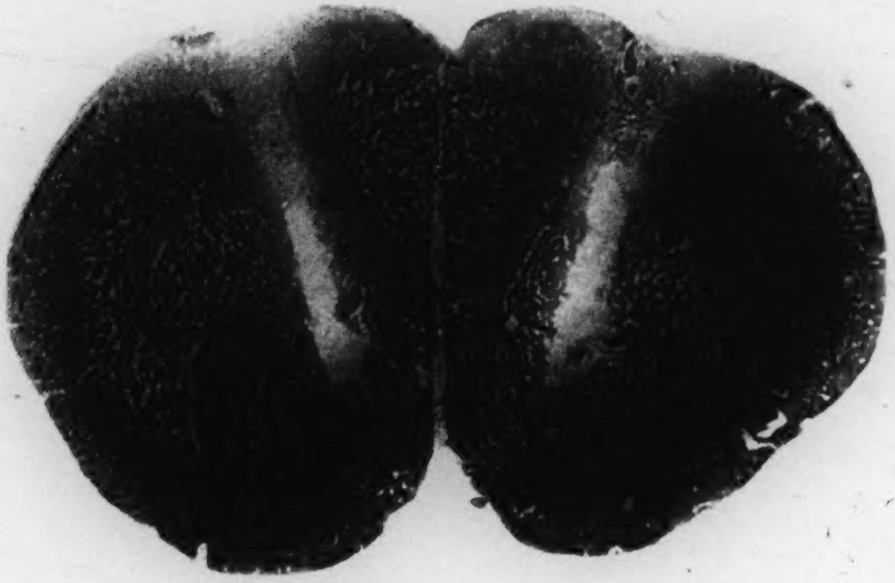
Appendix D. Histology protocols (continued)

Cresyl violet (Nissl) staining protocol

(courtesy K. Todd and E. Kwan)

70% ethanol	5 min
2 x 95% ethanol	5 min; 5 min
2 x 100% ethanol	5 min; 5 min
Hydrate:	
2 x xylenes (2 separate containers)	5 min; 5 min
2 x 100% ethanol	5 min; 3 min
2 x 95% ethanol	2 min; 2 min
70% ethanol	3 min
Distilled water	2 washes
0.5% cresyl violet (filter before use)	~60-90 sec
running tap water	until no excess
Dehydrate:	
70% ethanol	3 min
2 x 95% ethanol	2 min; 2 min
2 x 100% ethanol	4 min; 6 min
2 x xylenes	5 min; 5 min
Coverslip with Permount	

Appendix E. A representative photomicrograph of bilateral cannulae tips for microinjection into the NAS shell. This coronal Nissl-stained slice is approximately 10.7-11.2 mm anterior from interaural zero according to the atlas of Paxinos and Watson (1986).



Appendix F. A representative photomicrograph of an electrode tip in the VTA. This coronal Nissl-stained slice is approximately 3.4-3.7 mm anterior from interaural zero according to the atlas of Paxinos and Watson (1986).